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Relationships of weather variables and cultural factors with gray leaf spot severity on maize with emphasis on the preliminary development of a disease prediction

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Relationships of weather variables and cultural factors with gray leaf spot severity on maize with emphasis on the preliminary development of a disease prediction model

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GENERAL INTRODUCTION

Thesis organization

This thesis is composed of three chapters. The first chapter consists of the general introduction. Included in this section is some general information and background on gray leaf spot (GLS), followed by disease symptoms, epidemiology of the pathogen and management techniques for controlling the disease. The next two sections are separate chapters that report original research conducted during the period of study. The thesis is summarized by a general conclusion. Each chapter contains pertinent literature citations.

Introduction

Gray leaf spot (GLS) is an important foliar pathogen of maize (*Zea mays* L.). The causal organism, the fungus *Cercospora zeae-maydis* Tehon & Daniels, was first documented on maize in Illinois in 1925 (37). The first epidemic of GLS in the U.S. was reported in Tennessee and Kentucky in the early 1940s (15). The disease was next observed in Virginia about 6 to 7 years later (33) and in South Carolina in 1963 (19). Another severe epidemic of GLS followed in the early 1970s in North Carolina (21). Since the 1970s the disease has spread to most of the maize-growing regions of the United States hence it has gained the title of "a disease on the move" (20). In Iowa GLS is a major problem in the southern half of the state (25).

GLS is also a global problem. It has been reported to cause serious yield losses in South and Central America (6, 7, 20), and in Central and Southern Africa (41, 42). Yield losses have been as high as 69% in Virginia (36) and in Iowa, yield increases due to fungicide use have been as high as 3,178 kg/ha (16).

The dramatic spread of GLS in the U.S. has been attributed to the adoption of minimum tillage practices and the continuous cropping of maize (22). These farming practices leave a large amount of maize residue on the soil surface. Several studies have shown that the pathogen survives in residue and produces conidia in subsequent growing seasons (10, 27, 28). The conidia serve as primary inoculum and infect leaves of maize plants.

Gray Leaf Spot symptoms

The first symptoms of GLS infection are small tan spots about 0.1 to 0.3 cm long. The spots appear water-soaked and a chlorotic halo is visible in bright light (25, 42). It is not always easy to identify early-stage GLS lesions. In the U.S., GLS lesions usually appear as early as the 6- to 8-leaf stage or as late as anthesis depending on the prevailing weather in a particular year (25). The lesions begin on the lower leaves and spread upward to other leaves. This pattern suggests that leaf age might be a factor in determining susceptibility but it is more likely that the disease is simply spreading up from the soil surface (4).

Within 2 weeks, GLS spots mature into necrotic, tan-colored rectangular areas bordered by the leaf's secondary veins. Mature GLS symptoms are very characteristic and distinctive. In wet weather, abundant conidia are produced, giving the lesions a gray color (25, 42). Mature lesions can be 1 to 6 cm long and 0.2 to 0.4 cm wide (20). Lesion color, size and shape are variable depending on the resistance of the hybrid to GLS and inbred lines (11, 13, 21, 42). As their number and size increases, the lesions coalesce to form extensive blighted areas and photosynthetic area is reduced (1). As a result, fewer carbohydrates are produced, stalk strength is compromised, and the plant becomes more susceptible to stalk

rots. Plants with poor stalk strength may become lodged before harvest making it impossible to mechanically harvest the plants and further contributing to yield loss (41, 42). In addition to infecting leaves, the fungus can induce lesions on leaf sheaths, husks, and on the stalk itself. On these non-foliar parts of the plant, the lesions have a dark purple color and elliptical shape (25).

The growth stage at which GLS infects maize is a critical factor determining yield loss (16). In addition, hybrid susceptibility (12, 23, 32, 35) and the amount of initial inoculum (10, 28) also play major roles in disease development. If the plant is severely affected by GLS during grain-fill, insufficient photosynthate reaches the developing kernels and kernel size is therefore reduced. Such a situation results in fewer bushels of grain per acre, and in the case of maize seed, also alters the proportion of small, medium, and large kernels in the harvest (16, 42). In general, leaf diseases are known to have a major impact in maize seed production (24).

Epidemiology of *Cercospora zeae-maydis*

Cercospora zeae-maydis survives on infested maize residue from one season to the next (22). The fungus overwinters as stromata in the sub-stomatal spaces of maize leaves. In spring long conidiophores grow from the stroma and out of the stomatal openings (4). Conidia then develop from the conidiophores during favorable weather (20, 42). Infested maize residue is the initial source of spores at the beginning of the growing season and this first crop of spores is termed the initial inoculum (42). The conidia are relatively large, 70 to 180 microns long and 5 to 6 microns wide. They are hyaline, long with a tapered base and may have 6- to 10 septa (20).

Infection of a maize plant can occur after a conidium lands on the host surface. If conditions are favorable the spore will produce one or more germ tubes, which develop appressoria (4). The appressoria can develop infection pegs over a stoma and grow into germ tubes, which enter the host tissue and colonize the space below the stomata (42). The latent period, from infection until a new crop of spores is produced, ranges from 14 to 28 days (4) depending on hybrid susceptibility and persistence of disease-favorable weather conditions. Generally, more susceptible hybrids have shorter latent periods (32, 42). Because of the long latent period, secondary cycles occur late into the grain-fill period and may not affect yield as much as the primary cycle. Both primary and secondary inoculum is dispersed mainly by wind, but also by rain splash (42). Within one growing season of maize in the mid-western U.S., the fungus can produce up to four secondary infection cycles, hence GLS is considered to be a polycyclic disease (22, 42).

Ideal conditions for the development of GLS epidemics include relative humidity (RH) greater than 90% (34) and temperatures between 22 and 30 degrees C (5). GLS has been reported to be more common and severe in low-lying areas such as valleys, where frequent fogs and mists provide extended periods of high RH and wetness (4, 34). The direct influence of leaf wetness on infection is not well understood. However, the most important factor in GLS infection, RH, (4, 38) is particularly important for spore germination and infection (38). A single spore of *Cercospora zeae-maydis* can produce more than one germ tube, each of which can form an appressorium. A study by Thorson & Martinson (38) showed that germ tube elongation was favored by prolonged periods of RH over 95%. There was also a direct and positive relationship between the length of time germlings (germinated spores) were kept at 95% RH and the number of appressoria that formed per germling.

It seems that long periods of wetness or free water, as would be produced by rainfall and dew formation do not favor the infection process but rather favor germ tube growth and formation of mycelium on the leaf surface (4, 38). Greenhouse studies by Beckman & Payne (5) have shown that continuous periods of wetness produced less disease than with intermittent misting. Sporulation generally occurs in the morning in the presence of free moisture and dispersal of spores occurs in the afternoon when the dew has dried off the leaf surfaces (25). The germlings manage to survive fairly well in unfavorable conditions such as RH below 95% and temperatures above or below the optimum, and can resume infection once favorable weather returns (38).

Disease management

Options exist for GLS control and are most effective when used in an integrated approach. These include crop rotation, residue management, use of resistant hybrids, timely planting, and fungicide sprays (29).

Both crop rotation and tillage are residue management techniques since they aim to reduce levels of infested maize residue. If maize is alternated with a non-host crop such as soybeans, the maize residue decomposes significantly before the next maize crop is planted thus leaving very little debri for the fungus to survive on. *C. zeae-maydis* is not known to infect hosts other than maize (42). Some farmers in the U.S., however, prefer to grow continuous maize since it often fetches a better price than soybeans and they can feed the grain and stubble to their livestock.

Tillage buries most of the infested surface residue. Studies in Ohio and North Carolina have shown that burial of maize residue accelerates its decomposition and hence

reduces the survival of the fungus (10, 27) and the fungus cannot produce spores in the next growing season. But on surface residue, which takes longer to decompose, the fungus can still survive to sporulate the following summer (10).

A single year of a non-host crop, coupled with the use of tillage results in the buried fungus no longer being able to sporulate in the next growing season (10). In reduced tillage systems that leave 35% or more of the soil surface covered with residue, gray leaf spot can be managed with a rotation of two or more years of non-host crops between maize crops. GLS severity has been found to increase as the percent cover of soil surface with residue increases, although the strength of the relationship varies with environmental conditions (10).

The processes influencing field-to-field spread of *Cercospora zeae-maydis* spores are still not fully understood (22). Although studies have been conducted on the influence of different tillage systems and residue levels on disease and airborne spore concentrations (10, 27, 28), it is unknown whether airborne spore concentrations differ significantly above fields subjected to different crop rotation schemes. For this reason, one of the objectives of my research was to compare airborne spore concentrations in neighboring fields that differ in their crop rotation schemes.

The use of moderately resistant hybrids is highly recommended for control of GLS (23, 35, 42). Resistance to GLS in maize is polygenic (2) thus making it difficult to develop highly resistant hybrids, but partial resistance in hybrids improved significantly during the 1990's (8, 11). There is sometimes a trade-off between high resistance and yield (12, 35). In the past, farmers in the mid-west preferred to plant the high yielding varieties but it was found that these varieties were also highly susceptible to GLS. In light of the fact that it will be unlikely that extensive tillage practices will be reintroduced, planting of moderately

resistant hybrids is a valuable management practice. So far, only two genetically distinct populations of *C. zeae-maydis* have been identified (40), but they do not appear to differ in virulence or aggressiveness hence the use of resistance is still a viable option.

It has also been shown that early-planted maize develops less GLS and suffers less yield losses than late-planted maize. This is because earlier planted maize has a good chance of achieving full grain-fill before GLS becomes sufficiently severe to influence kernel size (25, 34).

Disease prediction models and the use of fungicides

Using fungicides to control GLS can be highly effective and profitable. The most common product for controlling GLS in the U.S. is propiconazole (Tilt, Novartis Crop Protection, Greensboro, N. Carolina), a systemic fungicide. Until 1999, Tilt could be applied only until 50% of the plants had silked (24, 25). Often, at this stage it is difficult to predict how severely GLS will develop on the crop and hence unnecessary applications are often made on maize seed crops.

Many university extension programs issue their own set of fungicide application recommendations based on hybrid or inbred susceptibility, residue levels in field, favorable weather, a disease threshold, and whether the inbred will be de-tasseled (25, 39).

Fungicide applications to inbred lines used in seed production are often made without assessing disease risk. This is because inbreds are economically valuable and the price of application is easily recovered by the sale of one or two units (80,000 kernels) of seed (24). Another reason is that female inbreds usually have greater susceptibility than their resulting hybrid, so there is a greater risk of yield loss than in hybrids. Fungicides are not commonly

used on hybrid maize in Iowa, the main reason being that grain is not worth as much as maize seed. Hence it is risky to obtain a profit by application of fungicide to control a disease like GLS, and the probability of a profit depends on the price of grain. In other states in the U.S. and in other countries, fungicide use is more common for GLS control in grain production than in Iowa (41, 42).

A limiting factor in deciding whether or not to use fungicides is the inability to predict whether yield gains will exceed the expense of fungicide application. This is especially true in hybrid maize. It is clear that more specific guidelines are needed regarding fungicide application but information about the epidemiology of the disease is insufficient to develop such guidelines. A system that can predict disease severity should greatly help with decisions regarding whether and when to apply a fungicide.

Prediction models have been successfully developed and implemented in other economically important *Cercospora* leaf spot diseases. The main basis for the prediction of disease is a detailed knowledge of the effect of environmental parameters on the different steps of the infection cycle of a pathogen.

Jensen & Boyle (17) first investigated the effect of temperature, relative humidity, and rainfall on peanut leaf spot caused by two different *Cercospora* species. Their most important conclusion was that duration of favorable RH and temperature was critical in the development of the leaf spot epidemic They described a method whereby temperature/RH indices were used to predict disease severity and found that their predictions agreed well with the actual disease severities observed.

In 1974, Parvin, Smith & Crosby (26) developed and implemented a computerized advisory system for prediction of conditions favorable for development of Cercospora leaf

spot on peanuts in Georgia. Weather data were collected and summarized daily into a Temperature/RH index. The index indicated the favorability of conditions for disease and ranged from "extremely favorable" to "unfavorable". The advisory was an elaboration of the technique developed by Jenson & Boyle (17, 18). Parvin et al's advisory agreed very well with the disease warnings issued by the agricultural meteorologist in the area (26) and resulted in fewer fungicide applications in borderline cases.

Another peanut leafspot advisory was developed by a computerized agro-environmental monitoring system (AEMS) in Virginia. Phipps & Powell (30) experimentally evaluated the success of this advisory. Before the implementation of the spray advisory in Virginia, growers would spray their peanuts every 14 days which commonly required 6 to 7 sprays during the growing season. When the advisory system was tested against the 14-day spray schedule, it was found that yields did not differ significantly despite the occurrence of significantly more disease on the plots sprayed according to the advisory. As few as 1 to 4 sprays were used in the advisory system without compromising yield. As a result, growers would use on average 2.25 fewer applications per season, thus reducing the costs of fungicide application by almost 33% (30).

Another important conclusion that emerged from this work was that timing of fungicide application was much more crucial in preventing yield loss than the number of sprays (30). Added advantages of reducing fungicide sprays included less mechanical damage to plants, a reduction in severity of other diseases due to plant injury, less compaction of soil and less environmental pollution (30). This advisory has been extremely successful and has been widely accepted by most growers in the peanut growing areas of Virginia (31).

The advisory, referred to as 81-ADV, was further refined by Cu & Phipps (9) by incorporating Time Duration Values (TDV's) into the prediction model. Environmental conditions conducive to pathogen growth (infection, germination, and sporulation) were given weighted values (TDV's) based on each hour of occurrence (9). The prediction model was successfully tested in the field and was then incorporated into the improved advisory, referred to as 84-ADV. This resulted in the same number of sprays as in the 81-ADV but better disease control was achieved.

Other prediction models for the control of *Cercospora* diseases have also been developed and utilized quite successfully, such as in the Peanut Leaf Spot advisory in North Carolina (3) and the *Cercospora* leaf spot model for sugar beet (43). These prediction models are also based on the strong relationship between duration of favorable environmental conditions and disease severity.

Similarly one of the chapters in this thesis describes the quantification of the relationships between environmental and cultural factors with GLS severity. This model could be used for the eventual development of a prediction model for gray leaf spot severity on maize.

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RELATIONSHIPS OF ENVIRONMENTAL AND CULTURAL FACTORS WITH GRAY LEAF SPOT SEVERITY ON MAIZE

A paper to be submitted to Phytopathology

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Abstract

Gray leaf spot of maize (GLS) can be managed using fungicides, but there is a need for criteria to improve the efficiency of fungicide use. In order to improve the knowledge of factors influencing gray leaf spot severity, environmental and disease severity data were collected in southern Iowa at thirteen locations in 1998, and eleven locations in 1999. The variables measured included temperature (T), relative humidity (RH), leaf wetness (W), percent maize residue cover (RES), distance to nearest maize residue (DIST), planting date (PLANT), and previous crop (CROP). Three to eight maize genotypes differing in GLS susceptibility and maturity were planted at each location. Disease severity was assessed at two-week intervals on these genotypes. Weather variables were accumulated for four different periods during the growing season and along with cultural and disease data, were analyzed by a linear stepwise multiple regression for each period separately in order to determine which variables significantly contributed to GLS at the R4/R5 growth stage of maize. When only the 1998 data were used in the analysis, genotype susceptibility (RAT), planting date (PLANT), distance to nearest maize residue (DIST), wetness (W), and TDV

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(hours when both temperature and RH are favorable) had significant effects on disease severity in the model and the R^2 did not differ much between the four periods. The best-fitting model for the 1998 data had an R^2 of 62%. With both the 1998 and 1999 data combined, the same variables were significant except that residue cover (RES) became significant instead of distance to nearest maize residue (DIST) and the best-fitting model had an R^2 of 43%. The most useful model for disease prediction was based on both years of data and utilized only the weather variables from emergence to 2 wk before silking (R1). Strong linear relationships existed between GLS severity and genotype susceptibility, maize surface residue, planting date, and hours of favorable RH and temperature. This knowledge can be applied toward the development of a prediction model for gray leaf spot severity on maize.

Introduction

Gray leaf spot (GLS), caused by the fungus *Cercospora zeae-maydis* Tehon & Daniels, has gained recognition as an important disease of maize (*Zea mays* L.) in the U.S. (16), parts of South and Central America (5, 7, 16), and South and Central Africa (38). The first epidemic of GLS in the U.S. was reported in Tennessee and Kentucky in the early 1940s (13). The disease was next observed in Virginia about 6 to 7 years later (31) and in South Carolina in 1963 (15). A severe epidemic of GLS was reported in the early 1970s in North Carolina (18). Since the 1970s the disease has spread to most of the maize growing regions of the United States (16). In Iowa GLS is a major cause of yield losses in the southern half of the state.

Gray leaf spot can cause significant yield reductions. Control of the disease with fungicides has resulted in yield increases as high as 7,129 kg/ha (106 bushels/acre) in

Virginia (35) and 3,178 kg/ha (66 bushels/acre) in Iowa (14). The critical factors that determine extent of yield loss in GLS epidemics include the growth stage at which GLS infects the maize plant, genotype susceptibility, the presence of sufficient hours of favorable weather (environmental conditions), the amount of initial inoculum in the field as determined by tillage practices, and crop rotation (19).

Cultural factors. The adoption of minimum tillage practices combined with the mono-cropping of maize are thought to be responsible for the drastic increase in GLS epidemics throughout the maize cropping regions of the U.S. (16, 39). The pathogen survives well in maize residue on the soil surface (9, 25, 26) and GLS severity has been found to increase as the percent of soil surface covered with maize residue increases (10). Residue in neighboring fields can also serve as a potential source of inoculum (11, 39). Another cultural influence on severity of GLS is planting date. Later planted maize generally develops more severe GLS than earlier-planted maize because the fungus can undergo a greater number of secondary cycles thus causing greater leaf damage (23, 32, 39).

Weather variables. Relative humidity (RH) and temperature are key influences on GLS development (4, 36). The most important factor for germination of and infection by *C. zeae-maydis* spores is RH. Thorson & Martinson (36) have shown that RH >/= 95% is optimal for germ tube elongation and formation of appressoria. Furthermore, prolonged periods of high RH have been shown to result in high levels of the disease (29, 32). A temperature of 22-30 C is optimal for germination and growth of the fungus (4). The duration of periods of leaf wetness may also be important for GLS development, but its direct influence is not fully understood. It appears to play a variable role in different parts of the infection cycle. It has been shown that a location experiencing almost daily periods of high

RH and wetness during the Jul-Sept period developed GLS whereas another location, which experienced fewer days of prolonged high RH and wetness, did not develop any GLS (32). The study concluded that wetness may encourage spore germination and spore survival as has been shown by Beckman & Payne (3). In the greenhouse, long periods of wetness or free water favor germination (3), germ tube growth and formation of mycelium on the leaf surface (3, 36) whereas intermittent periods of wetness have been shown to favor infection and result in more disease (3). Conidia and germlings of the fungus can survive fairly well at unfavorable temperatures or humidity and can resume the infection process once suitable conditions return (36).

Gray Leaf Spot management. Management practices for GLS include a combination of partially resistant hybrids, crop rotation, residue management, early planting and the use of foliar fungicides.

Tillage is unfortunately not a long-term solution albeit an effective one because of the problem of soil erosion. The situation is further exacerbated by the reluctance of farmers to adopt a suitable crop rotation scheme because of a lack of marketing opportunities for other crops.

Another good disease control method is the use of resistant cultivars. But flexibility in genotype selection does not exist in seed production. For this reason, farmers would often prefer to plant a high-yielding variety even though it may be susceptible rather than a resistant variety having a lower yield potential. Furthermore, even the best partially resistant hybrids that are available can suffer significant yield loss (12).

Fungicide use for GLS control has increased because of the limitations of other management practices. Fungicides have been shown to be profitable in maize seed

production (20). At least one application of propiconazole (Tilt, Novartis Crop Protection, Greensboro, North Carolina) per season is common for maize grown for seed in the U.S. because the price of application can be recovered by the high value of the crop. Although it has been shown that fungicide use can also be profitable in grain production (21), it has not been possible to predict whether yield gains will exceed the expense of fungicide application. The same limitation applies to maize grown for grain in South Africa (39).

Current fungicide recommendations are based on crop growth stage, cropping history, tillage practices, cultivar susceptibility, general weather conditions and a disease incidence or severity threshold, but these guidelines are not systemized (6, 23, 37). If disease severity could be predicted in advance, a grower's risk margin would greatly be narrowed.

Prediction models for fungicide applications have been developed and implemented in other economically important *Cercospora* leaf spot diseases. For example, advisories for fungicide applications for the control of peanut leaf spot in Virginia and North Carolina have been successfully implemented (1, 27). Others include the *Cercospora* leaf spot model for sugar beet (34, 40). These advisories have used weather indices based on accumulated hours during which conditions are favorable for steps in the infection cycle of the pathogen.

The objective of this research was to establish the basis for a GLS warning system by describing the quantitative relationships between environmental factors, agronomic factors and GLS severity using an empirical modeling approach.

Materials and Methods

Weather data and agronomic data were collected from thirteen locations in 1998 and eleven locations in 1999 in southern Iowa (Figure 1). The locations were chosen based on

cropping history, previous history of GLS, and the willingness of growers to participate in the study. Locations included commercial seed production fields, hybrid strip trials, and research plots. The latitude and longitude of each location were measured using a hand-held battery-operated Global Positioning unit (Magellan GPS 4000, Magellan Systems Corporation, San Dimas, CA, U.S.A.). Data were collected throughout the growing season from mid-May to late September when the maize had reached the dent stage (R5). The locations were visited every 2 wk to download weather data and collect agronomic data.

Weather data. Three weather variables were measured at each location– air temperature, leaf wetness and relative humidity (RH). Weather stations nearest each location were chosen as the source of rainfall data. The distance between the rainfall data weather station and the locations for the study ranged from being on-site to as far as 25 miles.

A SPECWARE datalogger (Spectrum Technologies, Inc., Plainfield, Illinois, USA) was placed at each location to record instantaneous air temperature (degrees C) and leaf wetness (0 to 15 scale) at 30-minute intervals. The flat sensor grid of the datalogger was painted by a proprietary process using three coats of a latex-based paint (Bob Olson, Savannah, GA; 17). The latex paint has been shown to increase the sensitivity of the sensor to dew and mimics emissivity of leaves better than an unpainted grid (17). The datalogger was mounted on a wooden block angled at 45 degrees to prevent retention of water droplets on the grid surface. The block was attached to a steel pole with the datalogger at a height of approximately 1.4-m above the ground and the wetness grid facing north. The pole was staked into an unobstructed grassy area immediately adjacent to each field. The aim was to measure on-site weather conditions rather than within-canopy weather conditions since environmental conditions within a corn canopy can be extremely variable due to increase in

plant height over the growing season and because the leaves can physically interfere with wetness measurements. In addition, poles located within fields could have interfered with routine farming operations.

Because the dataloggers measured leaf wetness indirectly, it was necessary to calibrate the sensor measurements to the wetness observed on the maize leaves. The wetness dataloggers were calibrated on two mornings and two nights in the July to September period in both years (1998 and 1999) by placing 2 to 4 dataloggers at the edge of a maize field near Ames, Iowa. The sensors were deployed as described previously. Dew onset was recorded in the evenings (night calibration) and dew dry-off in the mornings (morning calibration). In the night calibration, the dataloggers were programmed to measure wetness every 30 min in 1998 and every 10 to 15 min in 1999. Ten plants were randomly selected from the center of the field and leaves that were at the height of the datalogger and facing the same direction and angle as the datalogger were observed carefully every 10 to 15 minutes. The time was recorded at the first sign of dew on the upper leaf surface in at least 50% of the plants checked. In the morning calibration, the maize leaves were observed at the same intervals and the time was recorded when all the visible dew had dried off the leaf surface in at least 50% of the plants checked. Data from the dataloggers were compared to the visual observations to determine logger readings corresponding to dew onset and dew dry-off. Based on these data, any sensor reading greater than zero was recorded as a wet period.

RH was measured by placing a HOBO datalogger (Model RH Stowaway, Spectrum Technologies) in 1998, or Model H8 Pro Series (Onset Computer Corporation, Bourne, MA in 1999) inside a radiation shield (Spectrum Technologies) attached on the opposite side of the pole from the temperature-wetness datalogger at the same height. The HOBO measured

RH at 15-min intervals.

Rainfall data were obtained from measurements made at official weather stations nearest to each location. This information was available in the monthly issues of the Iowa Climate Review (Iowa Department of Agriculture and Land Stewardship). The daily rainfall amounts were accumulated for appropriate intervals during the growing season for each location.

Agronomic data. Tillage practices, cropping history, planting dates, and hybrids/inbreds planted all varied among locations.

At each field, residue cover was measured on the first visit in May, using the line transect method (22). A 16-m measuring tape was strung diagonally across the rows of corn. At every 0.3-m interval, the presence or absence of any maize residue at least 0.24 cm in diameter was recorded at 50 consecutive intervals were recorded for presence or absence of maize residue. The total number of intervals at which residue was found, were added and this gave the residue count. Three transects were sampled in each field, and the average residue count was then determined. The percentage of soil surface covered with residue was calculated by dividing the average number of counts by 50 and then multiplying by 100. Residue cover was a quantitative way of including tillage practices into the analysis. For fields that had no maize residue on the surface, the distance to the nearest source of surface maize residue was estimated visually.

At each site, the date on which the maize was planted was recorded as day of the year. In 1998, planting dates ranged from 23 April (day 112) to 24 June (day 182), and in 1999, from 27 April (day 117) to 27 May (day 147).

The crop (soybeans or maize) that had been planted in the field the previous year was noted. A previous maize crop would have contributed to the amount of surface maize residue present in the following year.

At each location, three to eight maize genotypes (hybrids and/or inbreds) varying in GLS susceptibility were planted by the cooperating grower. The genotypes also varied in the number of days to maturity. The genotypes were each given a GLS rating based on previous information (a scale of 1 to 9 with 1 being most susceptible and 9 being least susceptible). In this study, the GLS ratings ranged from 2 to 7 and maturities ranged from 98 to 119 CRM. Plot size ranged from 2 rows by 5m, to 12 rows by several hundred meters. Genotypes were not in replicated plots. All the locations did not have the same hybrids and/or inbreds planted but some genotypes were planted in more than one location.

Disease severity was visually assessed approximately every 14 days at the same time that weather data was downloaded from the dataloggers. Before taking any disease severity ratings in the field, a computerized disease assessment program known as CornPro (version 2.0) developed by Nutter & Litwiller (24) was used as a training tool for disease assessment. The program simulated GLS on corn leaves and the trainee was required to assess GLS severity on each leaf. The trainee's accuracy was determined at the end of the exercise by a graph of errors, which showed any over and under-estimations of actual disease, and a linear regression coefficient.

In the field, ten plants from each genotype were randomly selected from the middle of each plot and disease severity was visually assessed as the percentage of the whole ear leaf that was diseased due to GLS. Standard diagrams were used as reference (24). Prior to ear leaf emergence, presence or absence of GLS lesions was noted on whole plants. The growth

stage (30) of each hybrid or inbred was also noted at every assessment date during the growth season.

At the Iowa State University Southeast Iowa Research Farm (SERF) near Crawfordsville, plots were established with different tillage treatments and planting dates in a 25-m by 110-m plot in both 1998 and 1999 to make observations on GLS severity under different agronomic conditions. This plot had been continuously cropped with maize since 1992. Three hybrids of different susceptibilities were planted in adjacent tilled (fall chisel/spring disk) and non-tilled strips at two planting dates which differed by an interval of about 20 days. The hybrids ranged in susceptibility to GLS. Pioneer hybrid 3394 had a rating of 2 and was most susceptible, Pioneer hybrid 3489 had a rating of 4, and Pioneer hybrid 3335 had a rating of 5. In 1998, the early planting was 11 May (day 131), and the late planting was 27 May (day 147). In 1999 the early planting was 3 May (day 123), and the late planting was 19 May (day 139).

Data organisation. Previous studies have identified the optimum temperature and RH ranges for growth of and subsequent infection by *C. zeae-maydis*, but specific infection periods during the growing season have still not been defined. It was therefore appropriate to calculate the cumulative hours of conditions favorable for infection. Indeed, some studies have experimentally shown that cumulative duration of favorable conditions plays a pivotal role in infection and GLS development. For example, Thorson & Martinson (36) found that more appressoria formed with an increase in the number of hours spent at 95% RH. In addition, other models have successfully utilized this approach for disease prediction (1, 8, 34). Thus the following variables were derived from the actual measured variables: hours of

leaf wetness (W>0), hours with RH >/= 95% (RH95), and hours with temperature in the range of 22-30 C (T). An index, termed as the Time Duration Value (TDV), borrowed from the peanut early leaf spot model (8) was derived from RH and temperature. TDV was defined as the number of hours of conditions favorable for infection (in which both RH >/= 95 and 22</=T</=30 occur simultaneously). The above mentioned variables were accumulated for the following periods during the growing season: Hours from emergence to R5 (Period 1), Hours from 2 weeks before R1 (silking) to R5 (Period 2), Hours from 2 weeks before R1 to 2 weeks before R5 (Period 3), and Hours from emergence to 2 weeks before R1 (Period 4). These four periods were chosen because they were believed to be relevant to GLS development at the grain-fill period and also relevant to timing of fungicide applications.

Stepwise multiple linear regression was used to identify the mathematical relationships between the dependent variable (GLS severity at R4/R5 stage) and the several independent variables (derived weather variables, rainfall, agronomic and cultural factors). In a stepwise regression procedure, the independent variables are added one by one to the model if the F-statistic is significant for that particular variable. After the addition of each variable, all the variables which do not produce a significant F-statistic are removed (33). A default significance or p-level of 0.15 was used as the basis for the addition of a variable as well as for the retention of an added variable. The "proc reg corr" and "model" statements were used in the data analysis.

Disease severity at R4/R5 stage (D3) was chosen as the variable to be predicted because it has been found that disease severity assessment at this stage is optimum for yield loss estimation (14). A number of different combinations of variables were analyzed for their importance in the model. For each combination, a linear stepwise regression was run in SAS

for each period (period 1, 2, 3, 4) separately to determine whether the R^2 and the significant variables differed between the four periods. Listed below are the different combinations of variables that were analyzed by the linear stepwise regression procedure. There were 80 observations each in combinations 1 to 6, except for combinations 7 and 8, which had 180 observations.

The following independent variables were analyzed for their importance in GLS severity at the R4/R5 stage (D3) using the 1998 data:

Combination 1. Genotype relative maturity (MAT), genotype susceptibility (RAT), percent residue cover (RES), planting date (PLANT), distance to nearest source of maize residue (DIST), amount of precipitation during the four periods (RN1, RN2, RN3, RN4), and cumulations for RH95 (RH951, RH952, RH953, RH954), for temperature in the 22-30 deg C range (T1, T2, T3, T4), and for presence of wetness (W1, W2, W3, W4).

Combination 2. Combination 1 variables, and D1 (GLS severity at R1/R2) was also included as an independent variable in the analysis.

Combination 3. Combination 2 except that RH95 and T from periods 1 to 4 were removed and replaced by TDV (hrs when both RH and T are favorable) from periods 1 to 4 (TDV1, TDV2, TDV3, TDV4).

Combination 4. Combination 3 but without D1

Combination 5. Combination 1 but excluding DIST, to see whether RES would become significant on its own.

Combination 6. Combination 1 but excluding RES to see whether DIST would be significant on its own.

Combinations 1 through 6 all used only the 1998 data for predicting disease, but combinations 7 and 8 listed below used both 1998 and 1999 data combined in order to predict disease outcomes.

Combination 7. Combination 3 (TDV, D1) using data from both 1998 and 1999.

Combination 8. Combination 4 (TDV, no D1) using data from both 1998 and 1999.

The R^2 (coefficient of determination) and the variables significant at $p = 0.15$ were noted for each period in each model. For the regression equations from the most useful combinations, scatterplots were constructed to compare predicted disease to the actual disease that was observed. Equations based on 1998 data were used to predict 1998 and 1999 disease severities. Equations based on 1998 and 1999 data were used to predict 1999 severities.

Results

Wetness sensor calibration. The sensor wetness readings did not correspond exactly to visual observations of dew onset and dry-off in most cases. Generally, a particular sensor did not respond consistently at each calibration event (Appendix Table 1 and Appendix Figures 1 to 4). All four sensors responded early to dew dry-off with some as much as 75 minutes early and others only 15 minutes early. A similar pattern was observed in the dew onset events, with three of the four sensors recording dew onset 10 to 50 minutes early, and one sensor recording dew onset 60 minutes late. Based on these results, it was determined that the smallest error occurred if sensor readings of greater than zero were regarded as wet. Using this criterion, wetness periods were likely underestimated by 0-1 hr/day.

Disease variability due to cultural factors. At Crawfordsville (SERF), disease

severity was higher on the susceptible maize genotypes with hybrid 3394 having the most disease later in the growing season. In both years, GLS severity was greater in the no till strips where maize residue cover was 98% as opposed to the till strips where residue cover was about 62% (Figures 2 and 3). Also, the late-planted maize had much more overall disease at R4/R5 than the early-planted maize in both years. The differences in disease severity between till and no-till and early versus late planted were more pronounced on the more susceptible hybrids.

Disease variability among locations. Gray leaf spot severity on the same hybrid varied considerably in different locations (Figure 4A and Figure 5A). For example, in 1998, Pioneer 34T14, which is very susceptible with a rating of 2, had GLS severities ranging from 2% in Atlantic to 18% in West Branch, and the moderately resistant Pioneer 33G26 had severities ranging from 0.5% in Atlantic to 4.5% in West Branch (Fig 4A). Atlantic was a location in western Iowa whereas West Branch was in eastern Iowa. Both Anamosa (Anam) and West Branch (Wbra) in eastern Iowa had high levels of disease compared to the other three locations. The number of hours of favorable weather conditions at the five locations in 1998 were also compared (Figure 4B). Anamosa and West Branch had among the greatest hours of RH over 95% and Atlantic and Stuart, both in western Iowa, had the least. The trend with TDV's was a little different with Centerville having the highest number of TDV's, followed closely by West Branch. Anamosa had the least number of hours of TDV's even though it had among the highest disease.

In 1999, most of the same locations were compared for the amount of GLS on a number of hybrids ranging in their susceptibility to GLS (Figure 5A). The results were similar to those of 1998 with the susceptible hybrids like 33A14 and 35N05 at West Branch

developing the most disease and the same hybrids at Audubon (in western Iowa) and Centerville having comparatively much lower disease levels than West Branch. Keota had the highest disease severity. The moderately resistant Pioneer 33G26 had low levels of GLS at all the locations in both 1998 and 1999. The locations which showed high levels of disease on the susceptible hybrids (Keota and West Branch) had correspondingly greater numbers of hours of RH > 95%, and TDVs. But there was not such a great difference in the TDV values among the locations as there was in the RH95 values. Centerville and Oskaloosa both had much lower hours of RH95 and also had the lowest amount of disease (Figure 5B).

Regression analysis. In most of the combinations of variables tested, genotype susceptibility (RAT), planting date (PLANT), distance to nearest source of maize residue (DIST), wetness (W), rainfall (RN), and TDV (RH and T both favorable) were significant. The signs of the coefficients of all the variables tended to conform as expected, except for wetness and rainfall. The wetness variable always had a negative coefficient indicating that longer durations of wetness were associated with less disease. The rainfall coefficient sign was not consistently positive or negative in the analyses.

The R^2 did not differ much between the four periods in any of the combinations analyzed (Table 1). Adding D1 to the model (combination 2 versus 1) did not improve the R^2 to any great extent and generally the same variables were significant in both cases (Appendix Tables 2 and 3). This trend was also consistent in combination 4 versus 3 where RH95 and T were replaced with TDV (Appendix Tables 4 and 5).

Whenever D1 was used in the analysis, it was almost always significant for all the four periods but did not improve the R^2 to any notable extent. The replacement of RH95 and T with TDV (combination 3 versus 1) did not have much effect on the R^2 and the other

variables remained significant (Appendix Tables 2 and 4).

In the models based on the 1998 data (combinations 1 to 6), distance to nearest maize residue (DIST) was significant in the model but percent maize residue cover (RES) was not. When DIST was excluded from the analysis, it was found that RES became significant but the R^2 decreased a few points (combination 5), and when RES was excluded, DIST became significant and the R^2 was improved slightly (combination 6). When both 1998 and 1999 data were combined and analyzed together, the R^2 decreased somewhat but RES was significant and DIST was not (Appendix Tables 8 and 9).

The scatter-plots (Figures 6, 7 and 8) show the variation between actual and predicted GLS severity. In general, in all the scatterplots, there was a tendency to over-estimate disease when actual disease levels were low and underestimate disease when actual disease levels were high. This effect was especially pronounced in the model based on both years of data (Figure 8). A linear regression was run on each scatterplot in order to assess the prediction ability of that model. The R^2 was 49% and the slope was 1.20 when the model from the 1998 data was used to predict 1998 disease (Figure 6). When the same model from the 1998 data was used to predict 1999 disease the R^2 was less than 1% (Figure 7). With the model based on both years of data to predict both years of disease, the R^2 was 43% with a slope of 1.03 (Figure 8).

Discussion

Wetness sensor calibration. In general, the wetness sensors tended to detect dew before it was actually visible to the naked eye. But they also tended to dry off quicker than the maize leaves. Thus the duration of wetness recorded per day would tend to be a little under-estimated. However, this level of error was deemed acceptable.

Cultural factors. Genotype susceptibility, planting date, and maize residue were clearly demonstrated as important factors in GLS development. The effect of genotype susceptibility (RAT) was clear in the experiments at Crawfordsville (Figures 2 and 3), at the other locations (Figures 4 and 5), and in the regression analysis. In both years at Crawfordsville, disease at growth stage R4 was much greater on the late- planted maize than the earlier planted maize and planting date (PLANT) was a significant variable in nearly every regression equation. The late-planted maize tends to develop more severe levels of GLS since GLS is a late-season disease and late-planted maize experiences longer durations of high inoculum levels. The disease severity differences between the no-till and till strips are very informative considering that the strips were adjacent to each other and inoculum could have easily spread between plots. Residue cover (RES) or distance to maize residue (DIST) were also significant in each regression analysis. These results further reinforce the importance of tillage in one field even if the neighboring fields have not been tilled.

Variation in GLS severity among locations. The data from Figure 4 and 5 further support the fact that GLS can vary significantly on the same genotype if duration of favorable weather conditions is different. In both 1998 and 1999, the susceptible hybrids at West Branch developed much greater disease severities than those planted in the western part of the state. This difference related to the weather conditions in eastern and western Iowa. West Branch and Anamosa, both in eastern Iowa, had the highest number of hours of favorable relative humidity in 1998 and hence also had the highest levels of disease. The relationship between TDV and GLS severity was not as strong as that between RH and GLS severity. This suggests that disease development is less sensitive to temperature than it is to relative humidity. It has in fact been observed that high temperature and low rainfall are not

as limiting on GLS severity as are micro-climatic factors such as RH (2). However, the relationships between RH, TDV, and GLS severity are confounded with other variables that differed among locations.

Regression analysis. The stepwise regression gave reasonably high R^2 values for the models considering that field data were used for the model development as opposed to a laboratory situation where conditions can be strictly controlled. The analyses also identified the variables considered to be significant in the models.

Since the R^2 values did not differ much between periods in any of the eight combinations, it would be advantageous to use only period 4 variables for the model. This period would allow the earliest disease prediction to be made and this is very important with regards to fungicide application decisions for gray leaf spot. The coefficient signs for the significant variables were as expected, except for wetness, which had a negative coefficient as pointed out earlier. This means that longer wetness durations were associated with lower disease severity. This is unusual for a foliar disease, but is consistent with other studies conducted on *C. zeae-maydis*. The role of wetness in GLS development has not been established. In fact, a study by Thorson & Martinson (37) showed that increased wetness discouraged the formation of appressoria by *C. zeae-maydis* germlings and rather, favored the development of mycelium on the leaf surface. In another pathosystem involving *Cercospora beticola* on sugar beet, the germ tube has been found to grow toward stomata only in the absence of free water and it has been therefore suggested that the tropism may occur in response to a water-vapor gradient (28). The same may hold true for *Cercospora zeae-maydis* on maize. Another possible explanation may be that when free water is present on the leaf surface, the fungus can no longer "sense" the leaf surface topology near the

stomata and hence penetration does not occur. Therefore the presence of wetness or moisture on the leaf surface may actually inhibit the infection process.

Including D1 in the 1998 models did not improve the R^2 much and the same variables were significant except rainfall (RN), which was no longer significant. If D1 were to be incorporated into a predictive model, it would delay disease predictions by another two weeks compared to a model using only the variables measured for period 4.

The reason for rainfall not being significant might be explained by the fact that rainfall is indirectly a measure of wetness so the wetness variable was a sufficient representation in the model. Also, rainfall was not measured on location, so its relationship with disease may not be strong. Ringer & Grybauskus (29) found that total rainfall observed throughout the full growing season did not necessarily correspond with highest levels of GLS. Rather, the amount of rainfall early in the season was more influential for the amount of GLS that developed. But in the models with both years of data combined, rainfall was still significant even after addition of D1 and the R^2 improved.

Replacing RH95 (hrs of favorable relative humidity) and T (hrs of favorable temperature) with TDV (hrs when both RH and T are simultaneously favorable) improved the R^2 slightly and did not change the variables that were significant. Temperature was not significant in any of the periods and relative humidity followed this same outcome except that it was significant a few times. In contrast, when TDV was used in place of RH95 and T, it turned out significant every single time. It is important to note at this point that RH95 represents all the hrs when RH was favorable for the fungus regardless of whether temperature was favorable or not, and T represents all the hrs when temperature is favorable regardless of whether RH is favorable or not. Based on this point, it seems that the fungus

must require conditions when crucial weather variables such as RH and T have to be simultaneously favorable (as represented by TDV) and one single variable alone being favorable is not sufficient for growth of the fungus.

In the models based on both years of data, RES replaced DIST as the significant variable. The probable explanation for this is that RES and DIST are inversely related and with two years of data as opposed to just one year of data, the effect of RES was no longer overshadowed by the effect of DIST in the model.

The model derived from 1998 data did not accurately predict 1999 disease levels (Figure 7). This result demonstrates that more than one year of data collection is necessary for development of a robust model. The scatter-plots show that some of the predicted values had negative values for disease severity. This is primarily due to the variable DIST. Fields that had high values for DIST (which means that the field was further away from a neighboring inoculum source) often had negative values for predicted disease severity and resulted in great distortion in the linear distribution of actual versus predicted values as is shown in Figure 7. If DIST were to be used in a predictive model, it should be given a maximum value less than 50. Also, in order to develop a reasonably reliable prediction model, it is important to use more years of data.

The model obtained from period 4 of combination 8 appears to be most appropriate for disease prediction. Period 4 was chosen because as discussed earlier in this section, it is the time from emergence to 2 weeks before silking and variables measured in this time frame allows for the earliest disease prediction to be made. Secondly, combination 8 variables were chosen because they included TDV instead of RH95 and T since TDV appeared to be more

important than the other two variables, and lastly this combination used two years of data as opposed to one year of data.

This research has shown strong linear relationships between GLS severity and genotype susceptibility, maize surface residue, planting date, and hours of favorable RH and temperature. These relationships can be exploited to develop a predictive model for GLS severity. However, results indicate that somewhat better predictions might result from a non-linear modeling approach. Additional observations in the database should also contribute to the development of a robust predictive model.

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Table 1. Coefficients of determination (R^2) in percent, for multiple regression models tested for prediction of gray leaf spot severity.

	Period			
Combination	**1**	**2**	**3**	**4**
1	62	61	62	60
2	61	64	62	60
3	65	62	63	62
4	64	59	63	62
5	49	58	50	45
6	62	61	62	60
7	55	51	50	48
8	48	45	46	43

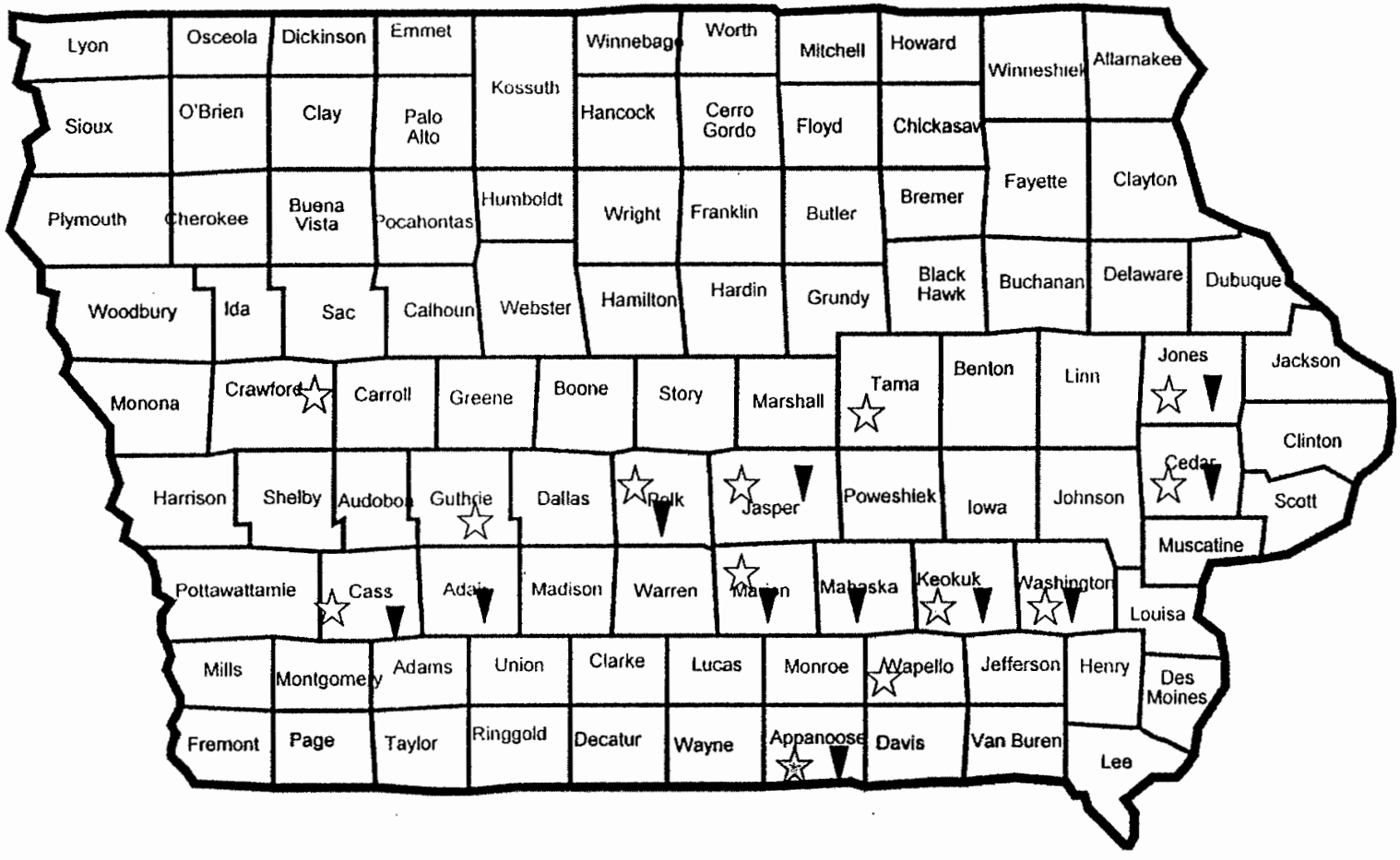


Figure 1: Map of Iowa showing the locations in 1998 and 1999 where data was collected.

☆ **1998 locations** ▼ **1999 locations**

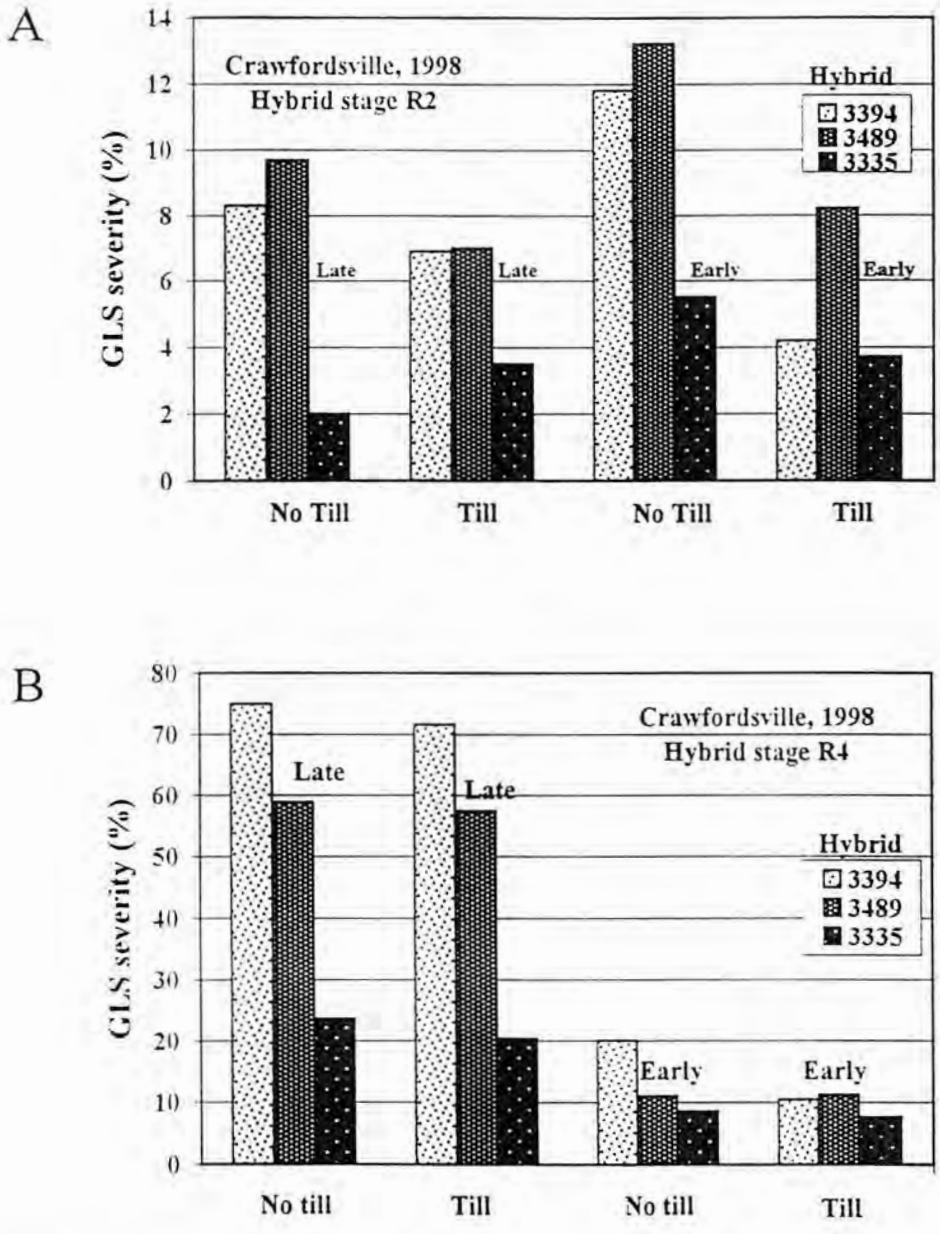


Figure 2: Effect of tillage and early planting on GLS severity observed on three hybrids varying in GLS susceptibility.
Pioneer 3394 was the most susceptible (2), followed by 3489 (4), and 3335 (5).
(A): At hybrid stage R2
(B): At hybrid stage R4

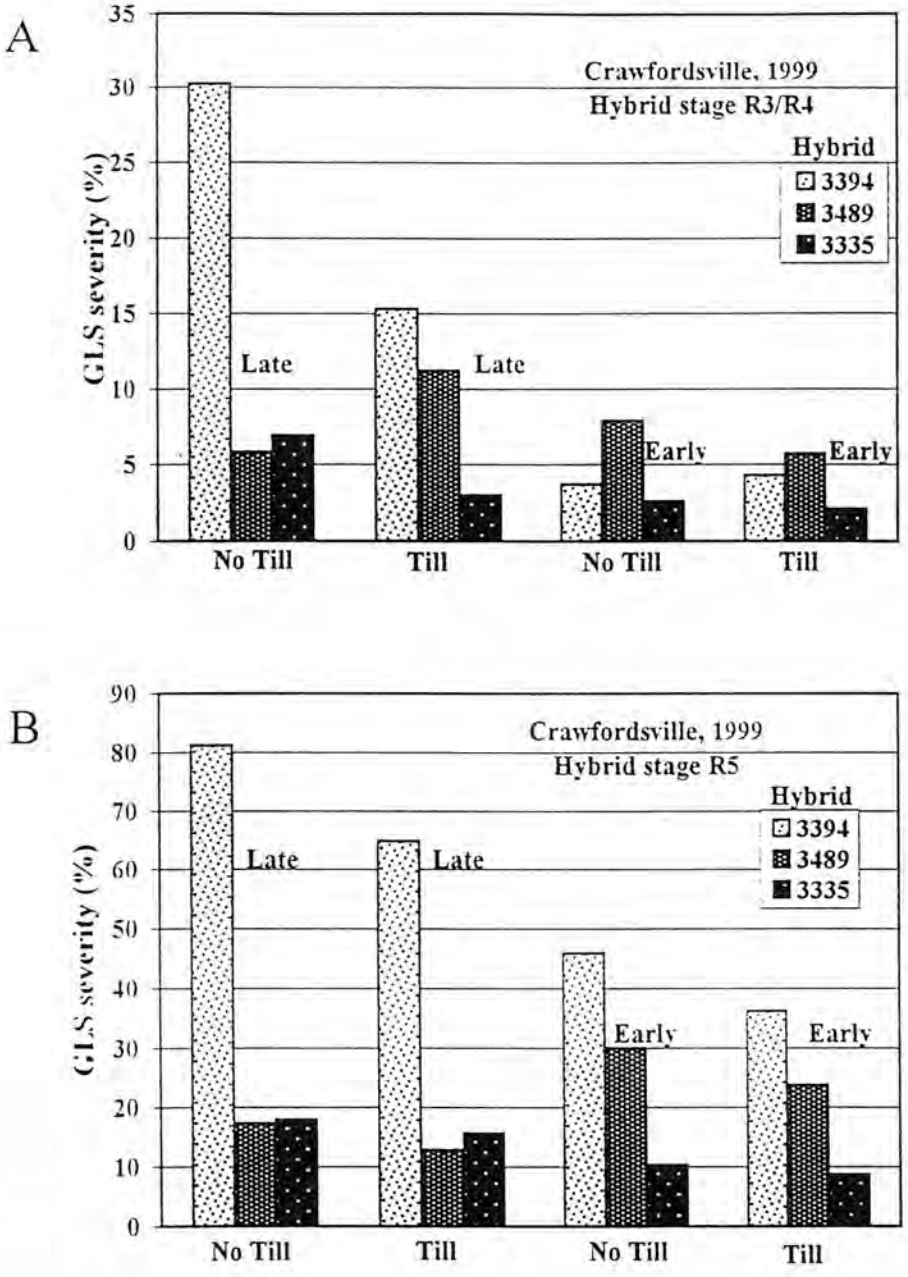
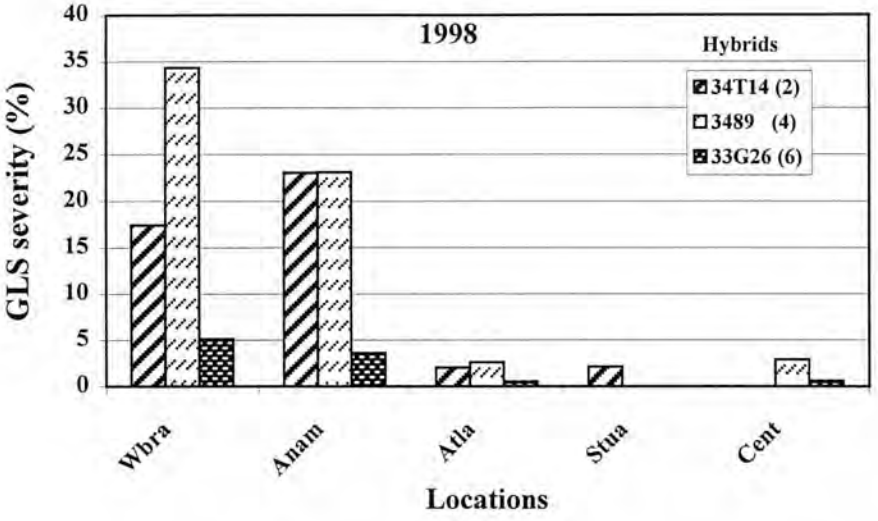


Figure 3: Effect of tillage and early planting on GLS severity in three Pioneer hybrids varying in GLS susceptibility in 1999.
Pioneer 3394 was the most susceptible (2), followed by 3489 (4), and 3335 (5).
(A): At hybrid stage R3/R4
(B): At hybrid stage R5

A



B

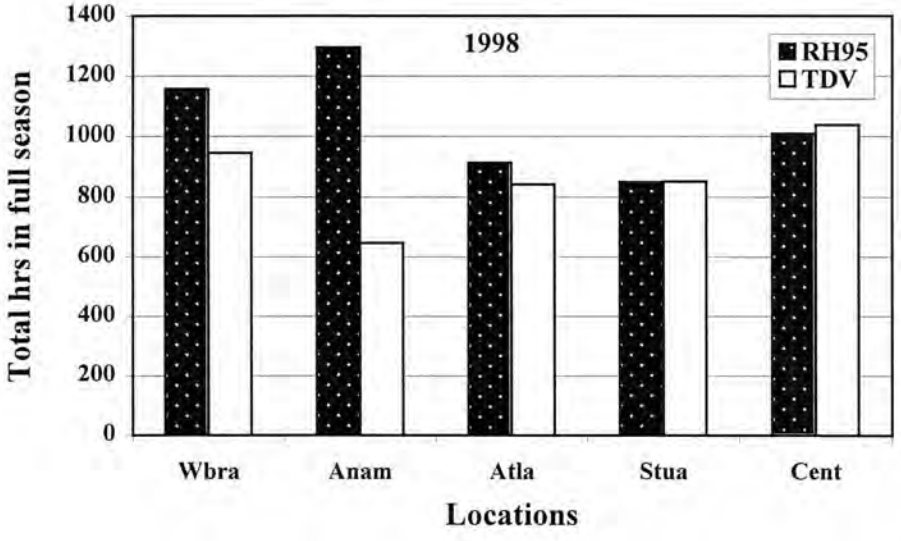
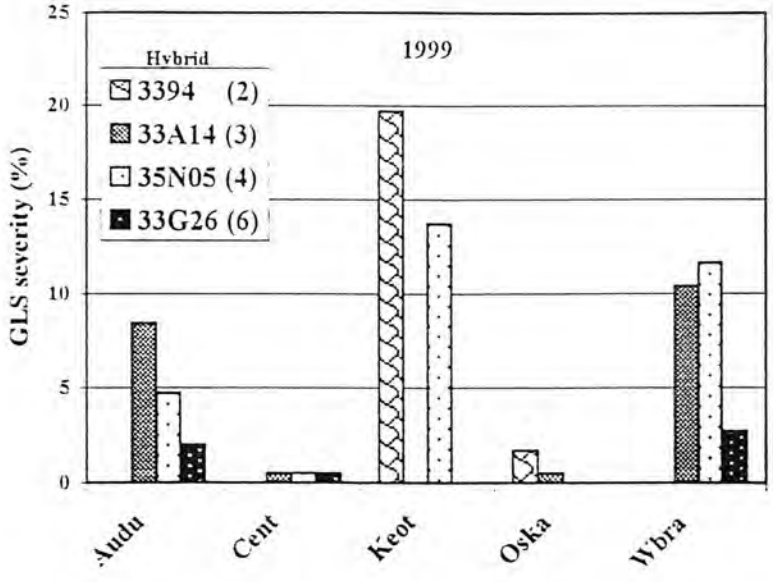


Figure 4 : (A) Variation in GLS severity in 1998, on three hybrids all planted at five different locations in Iowa.
(B) Variation in number of hours of RH95 and TDV between the same five locations in 1998.

A



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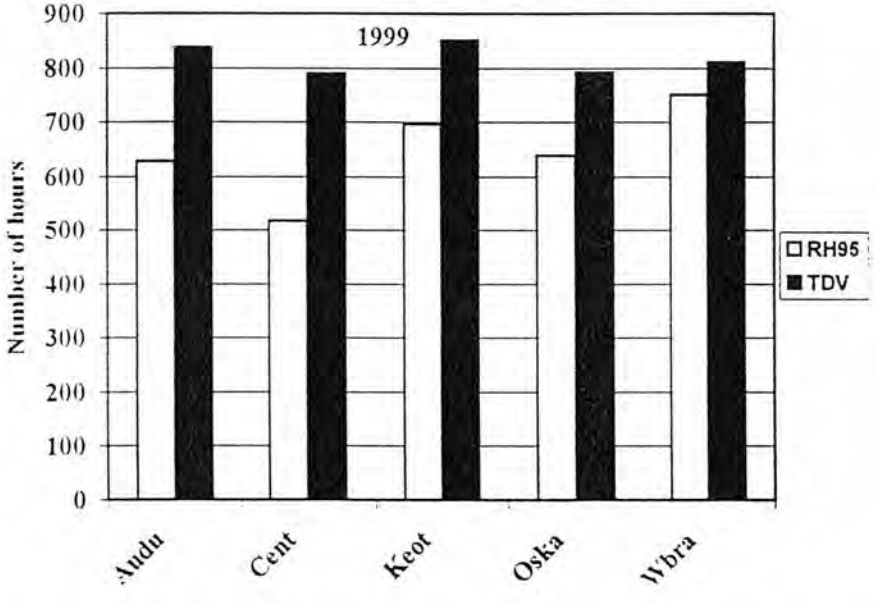

Figure 5. (A) Variation in GLS severity in 1999 on four hybrids all planted at five different locations in Iowa.
(B) Variation in number of hours of RH95 and TDV between the same five locations in 1999.

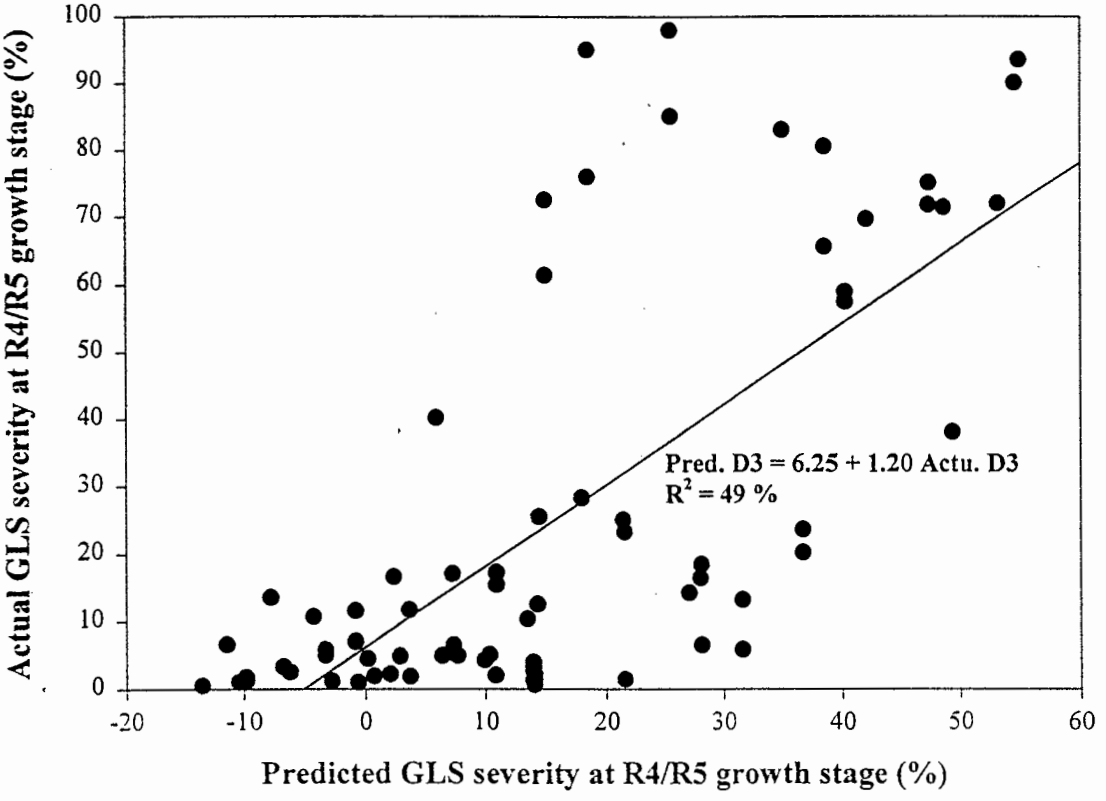

Figure 6: Scatterplot and simple linear regression of observed GLS severity in 1998 versus predicted GLS severity in 1998 based on the prediction equation from the 1998 data (period 4 of combination 4).

Note: See Appendix Table 5 for combination 4 variables.

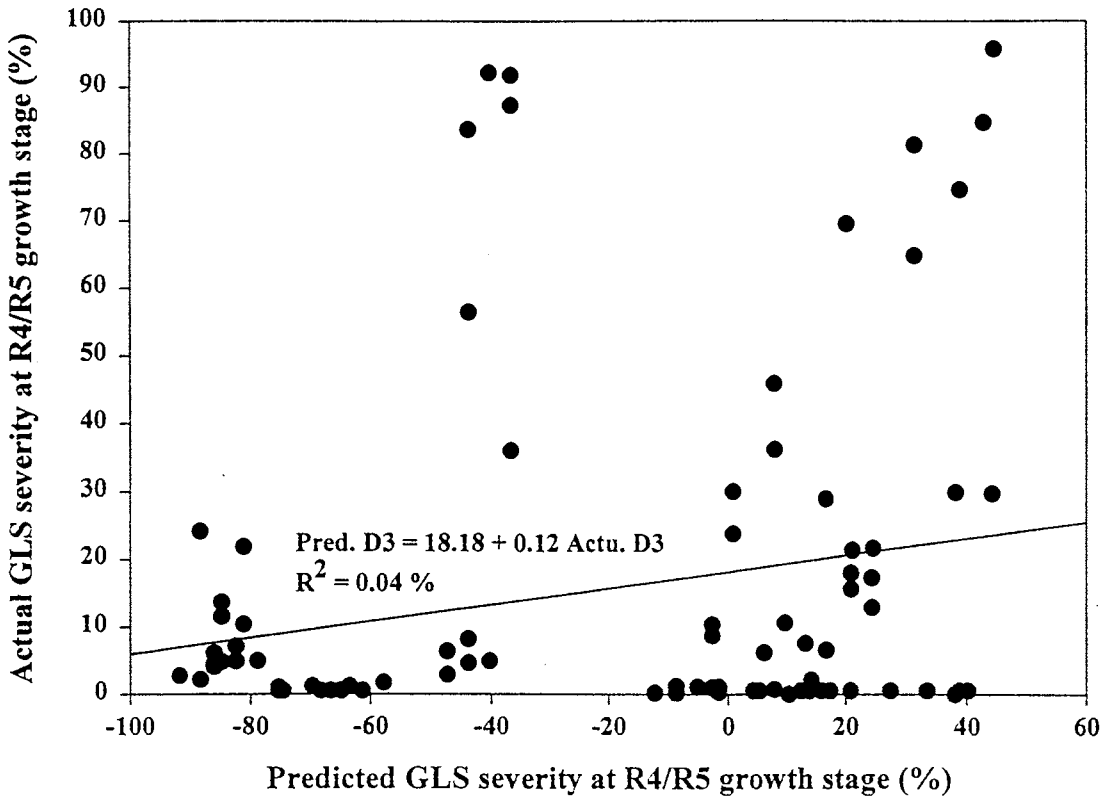

Figure 7: Scatterplot and simple linear regression of observed GLS severity in 1998 versus predicted GLS severity in 1999 based on the prediction equation from the 1998 data (period 4 of combination 4).

Note: See Appendix Table 5 for combination 4 variables.

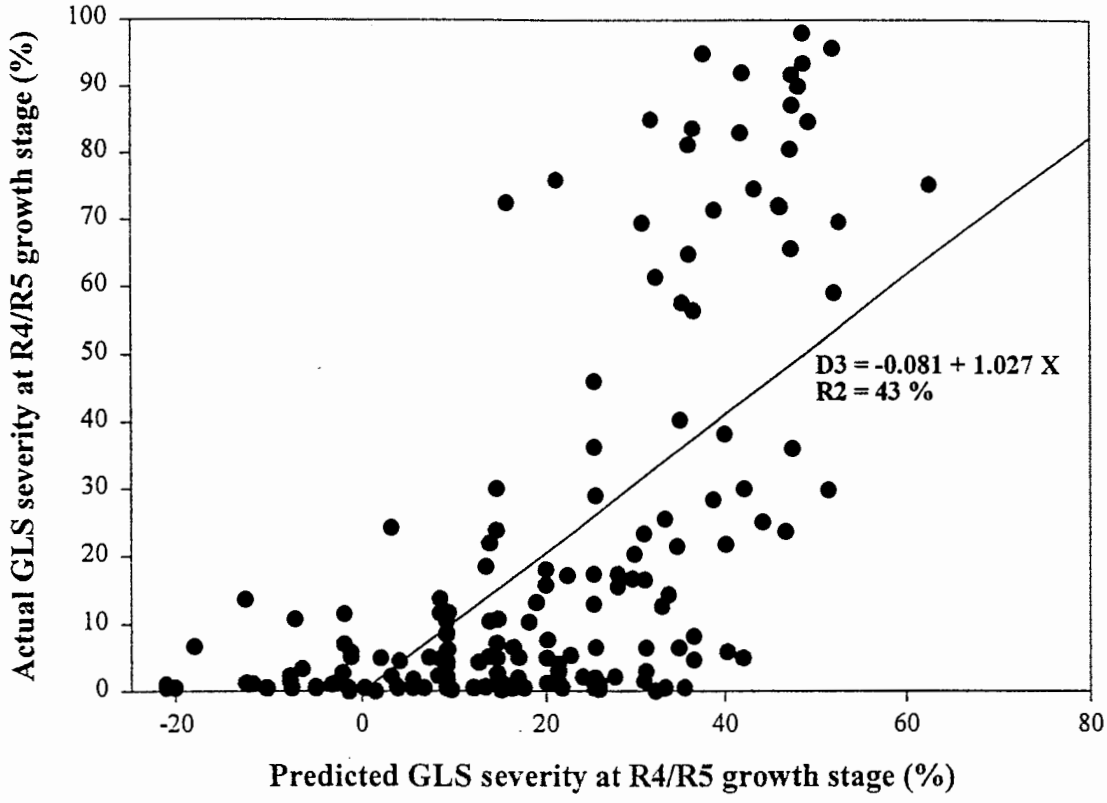

Figure 8 : Scatterplot and simple regression of actual GLS severity in 1998 and 1999 versus predicted GLS severity in 1998 and 1999 based on the prediction equation developed from both 1998 and 1999 data (combination 8).

Note: See Appendix Table 9 for combination 8.

CERCOSPORA ZEAE-MAYDIS AIRBORNE SPORE CONCENTRATIONS IN CORN FIELDS UNDER TWO DIFFERENT CROP ROTATION SCHEMES.

A paper to be submitted to Phytopathology

A. Bhatia [1] and G.P. Munkvold [2]

Abstract

Crop rotation is often recommended as a management practice for Gray leaf spot (GLS) of maize, but the airborne nature of its conidia may influence the efficacy of this practice in areas of extensive maize cultivation. Conidia of *Cercospora zeae-maydis* were sampled from air in two maize fields in 1998 and 1999 at the Southeast Iowa Research Farm (SERF) in Iowa. The two fields differed in their crop rotation schemes with one field planted with soybeans in the previous year and the second field planted with maize in the previous year. The spore samplers were operated for 24 hours at each 2-week interval from 24 June to 06 September. It was found that early in the growing season the fields did not differ significantly in their airborne conidia concentration but later in the growing season the maize-maize field had higher concentrations of airborne *C. zeae-maydis* conidia. Gray leaf spot severity was similar for both fields early in the season but became much higher in the maize-maize field later in the season around the R4 growth stage. The results support the efficacy of crop rotation for the management of GLS in SE Iowa.

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Introduction

Gray leaf spot, commonly referred to as GLS and caused by the fungus *Cercospora zeae-maydis* Tehon & Daniels, is a very important foliar disease of maize (*Zea mays* L.). The first epidemic of GLS in the U.S. was reported in Tennessee and Kentucky in the early 1940's (3). The disease was later found in Virginia about 6 to 7 years later (14) and in South Carolina in 1963 (5). A severe epidemic of GLS was reported in the early 1970's in North Carolina (7). Since the 1970's the disease has spread to most of the maize growing regions of the United States hence it gained the title of "a disease on the move" (6). In Iowa GLS is a major problem in the southern half of the state.

The adoption of minimum tillage practices and the monoculture of maize have been implicated in GLS epidemics (6, 15). Both these cultural practices contribute to the accumulation of large amounts of infested maize residue on the soil surface. *Cercospora zeae-maydis* is a residue-borne fungus (8) that survives in the form of stromata in the sub-stomatal spaces of maize leaves. In spring long conidiophores arise from the stromata and out of the stomatal openings. Conidia then develop from the conidiophores during favorable weather (15).

Several studies have examined the survival of the fungus under conventional versus reduced/minimum tillage. One study conducted in North Carolina showed that the fungus survived on both surface and buried residue for 6 months at one location, but only for 1 month on buried residue at another location (11). Another study from Ohio showed that the fungus only sporulated for 3-4 months after burial of residue but was able to sporulate on surface residue after 9 months (1). They also found that the buried fungus sporulated less than the fungus from surface residue. It is now accepted that there is a direct and positive

association between amount of infested surface maize residue and GLS severity although environment can greatly affect the strength of this association (1, 12).

The field-to-field spread of *Cercospora zeae-maydis* spores is still not fully understood (8). The conidia of this fungus can be carried by wind to neighboring fields and disease gradients have been characterized using point inoculum sources (2). Although studies have been conducted on the influence of different tillage systems and residue levels on the airborne conidial concentrations of *Cercospora zeae-maydis* (1, 11, 12), it is unknown whether airborne conidial concentrations differ significantly in neighboring fields under different crop rotation schemes. Furthermore, the scale dependence of spore dispersal gradients makes it difficult to extrapolate small-plot results to a commercial production setting. In south-east Iowa, GLS is widespread and most maize crops are planted where maize or soybeans were the previous crop. Maize planted into soybean stubble is usually within 100-m of *C. zeae-maydis* infested maize residue.

The efficacy of crop rotation in GLS management depends on the extent to which it influences airborne *C. zeae-maydis* inoculum levels. Thus the objective of this research was to compare airborne conidial concentrations of *Cercospora zeae-maydis* with GLS development in two fields under different crop rotation schemes in an area of intensive maize production in southeast Iowa.

Materials and Methods

Field locations. Sampling of airborne conidia of *Cercospora zeae-maydis* was conducted in two neighboring fields. In 1998 the two fields were approximately 20-m apart and separated by a grass strip about 10-m wide at the Iowa State University Southeast

Research farm (SERF) in Washington County, Iowa. In 1999, the two fields were about 70-m apart. Each field was approximately 2 ha in area. Each year, one field had been planted with maize the previous year (maize-maize field) and the second field had been planted to soybeans the previous year (soybean-maize field), thus the two fields differed in the amount and age of surface maize residue. GLS was present the previous year in the maize-maize fields and also was present throughout the area. In 1998, a mixture of maize hybrids was planted in the maize-maize field and Pioneer hybrid 34R06 was planted in the soybean-maize field, whereas in 1999 both fields were planted with the hybrid Garst 8481 Bt.

The percentage of soil surface covered with infested maize residue was determined in both fields in 1999 using the line transect method (9).

Spore sampling. In 1999, all four Rotorod samplers were sent for calibration (Sampling Technologies, Inc., Minnetonka, Minnesota, USA) before their use in the field. The Rotorod samplers were not calibrated before use in 1998 hence there was an error of 0 to 20% in the conidial concentrations due to the samplers not all operating at the same speed. Every two weeks from 26 June to 6 September in 1998 and 24 June to 1 September in 1999, two Rotorod spore samplers were placed in the center of each field approximately 15 m apart. The Rotorod samplers consisted of a small motor to which a steel rotating arm with retracting heads was attached. A clear plastic rod was attached to each end of the arm. The impacting rod surface was exposed during sampling and retracted back into the head when the sampling period was over. The Rotorod samplers were suspended from a curved steel pole so that the sampling rods were 1.5-m above the soil surface. A circular plastic shade shielded the spore samplers from rain. The two spore samplers in each field were connected to the same battery and cycling field timer (Model 30, Sampling Technologies Inc.). Before

connecting the samplers to the battery, the impacting surface of each rod was coated lightly with silicone grease so that conidia would adhere to the rod surface upon impaction. The timer was programmed to operate for 30 seconds every 10-min over a 24-hr period. At each sampling date the sampling began at approximately 12:00 and was stopped 24 hr later. The exact time that sampling was started and stopped was noted for each field at each sampling date. The two collector rods were transferred by forceps into a plastic storage vial (Sampling Technologies, Inc.). Sampling date and field location were recorded for each rod. The rods were then stored at 4° C until the conidia could be counted.

Counting of conidia. The conidia on each rod were counted by sliding a rod into one of the grooves of a clear plastic stage adapter (Sampling Technologies, Inc., Minnetonka, MN, U.S.A.) such that the impacted rod surface was on top. The conidia were stained with one drop of trypan blue in 10% acetic acid, and a 22 by 22-mm cover glass (#11/2, Sampling Technologies, Inc.) was placed on top of the rod surface to obtain a temporary wet mount slide. The number of *Cercopsora zeae-maydis* conidia in the area covered by the length of the cover glass and the width of the collector rod (1.52 mm) were counted under a light microscope at 200X magnification. Conidia were identified based on their morphological characteristics (6) and by comparing them with conidia from *C. zeae-maydis*-infected leaves. The spore count was then converted into the number of conidia per m^3 of air sampled. The amount of air sampled was calculated based on the impaction area, rotation speed of the samplers, and sampling duration.

Disease ratings. At each sampling date in 1999, 20 maize plants were randomly picked from the center of the field and checked for presence of GLS lesions. Once the ear leaf had developed, GLS severity ratings were also taken at each sampling date. GLS severity

was assessed as the percentage of the ear leaf that was covered by GLS lesions using a standard area diagram as a guide (10).

Results

Early in the 1998 season, conidial concentrations were very low and there was not much difference between the two fields. But later in the growing season, the field which had been planted to maize the previous year had higher concentrations of conidia than the field which had soybeans as its previous crop (Figure 1). Replication of spore counts was not sufficient to perform a statistical comparision.

In 1999, the maize-maize field had a maize residue cover of 78% and the soybean-maize field had a maize residue cover of 22%. Residue cover was not measured in 1998 but was very similar to the residue cover in 1999 since similar rotation schemes were used in both years. Conidial concentrations in 1999 had a similar pattern to conidial concentrations in 1998 early in the growing season, with conidial concentrations being very low. As the growing season progressed, the maize-maize field did not consistently have higher concentrations of conidia. The soybean-maize field generally had lower conidial concentrations on 21 July and 1 September but had higher concentrations on 5 and 19 August. At the end of the growing season, the differences in conidial concentrations between the two fields were at their greatest in both 1998 and 1999 with the maize-maize field having significantly higher concentrations.

Gray leaf spot lesions were not seen in both fields in 1999 in the first sampling period of 25 June. By the second sampling period, which occurred 2 weeks later, both fields had GLS lesions reaching up to the 12^{th} or 13^{th} leaf and the GLS lesions were much larger and

more numerous on the lower leaves in the maize-maize field. By the R1 growth stage, plants in both fields showed less than 1% GLS severity on the ear leaves. Later in the growing season, the plants in the maize-maize field developed much more severe GLS than did the plants in the soybean-maize field (Figure 2B).

Discussion

Despite the considerable difference in maize residue level between the two fields, airborne concentrations of *Cercospora zeae-maydis* conidia were very low and not different in both fields early in the growing season. These results contrast with those of Jenco (4), who showed that conidial release was significantly high during June and July at SERF in 1991 and 1992. There could be at least two reasons for these findings. It is quite possible that in these two particular years, weather was not favorable early in the season and so very little sporulation occurred early in the season. Or, the second explanation could be that due to our long sampling intervals (every two weeks), we might have missed sporadic sporulation events that occurred only on specific days.

Due to the long latent latent period of the fungus, the infection cycles early in the season may be more important than the collective infection cycles in a full season (13).

Conidial concentrations increased later in the season, when it is likely that secondary sporulation cycles were contributing. The time required for the fungus to sporulate on lesions varies from 2 to 4 weeks for *Cercospora zeae-maydis*, and is also affected by the environment (13). During August, the weather was apparently very favorable for the fungus and it produced abundant conidia, especially in the fields with higher residue levels. The origin of these conidia was likely a combination of residue and lesions, but the relative

contributions of these two sources cannot be determined. Similarly, conidia sampled in the soybean-maize fields probably originated from the year-old maize residue and from wind dispersal from other fields. Spore dispersal studies with *C. zeae-maydis* suggest that very few conidia are dispersed outside their field of origin (2), but in areas where with intensive maize production, this undoubtedly contributes some inoculum to soybean-maize fields.

As the season progressed, GLS lesions appeared earlier in the maize-maize field and there was greater and more severe infection of the lower leaves in these fields as compared to the soybean-maize field. Although differences in airborne spore concentrations between fields were relatively small during the early reproductive stages of the maize crop, this difference was apparently sufficient to initiate a stronger epidemic in the maize-maize field. By early September, disease severity in the maize-maize field was four-fold that in the soybean-maize field.

In addition to differences in airborne conidial concentrations, another factor possibly contributing to less disease in the soybean-maize field is the loss of viability of conidia during dispersal. In this study, viability of conidia was not evaluated. If a large proportion of the conidia sampled in the soybean-maize field had been blown in from other fields, their viability may have been lower, resulting in less disease as compared to in the maize-maize field.

High conidial concentrations in the maize-maize fields during the September sampling, were associated with the higher disease severity. Conidia released this late would contribute little to disease-related yield loss.

This study supports the use of crop rotation as a component of a gray leaf spot management strategy. Fields with soybeans as the previous crop generally had lower airborne

concentrations of *C. zeae-maydis* conidia and developed less GLS by the R4/R5 growth stage than fields where maize was the previous crop.

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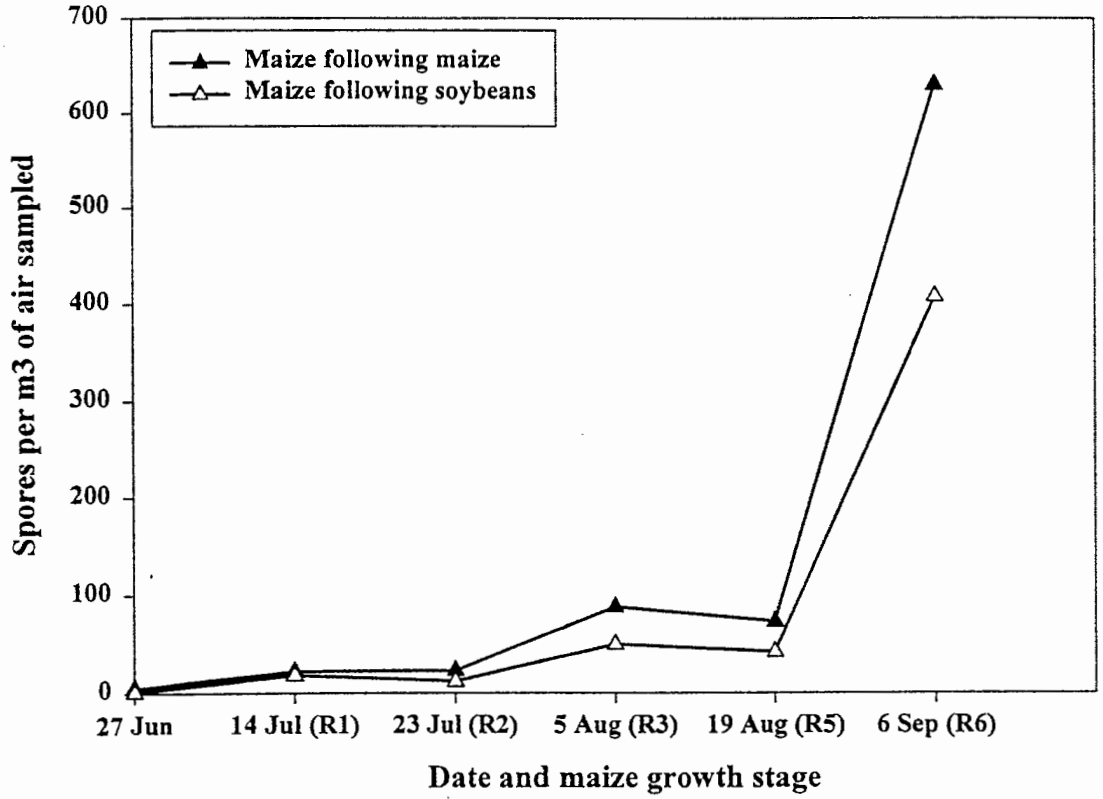


Figure 1: ***Cercospora zeae-maydis*** **spore concentrations from June to September in corn fields at the Southeast Iowa Research Farm (SERF) in Iowa, 1998.**

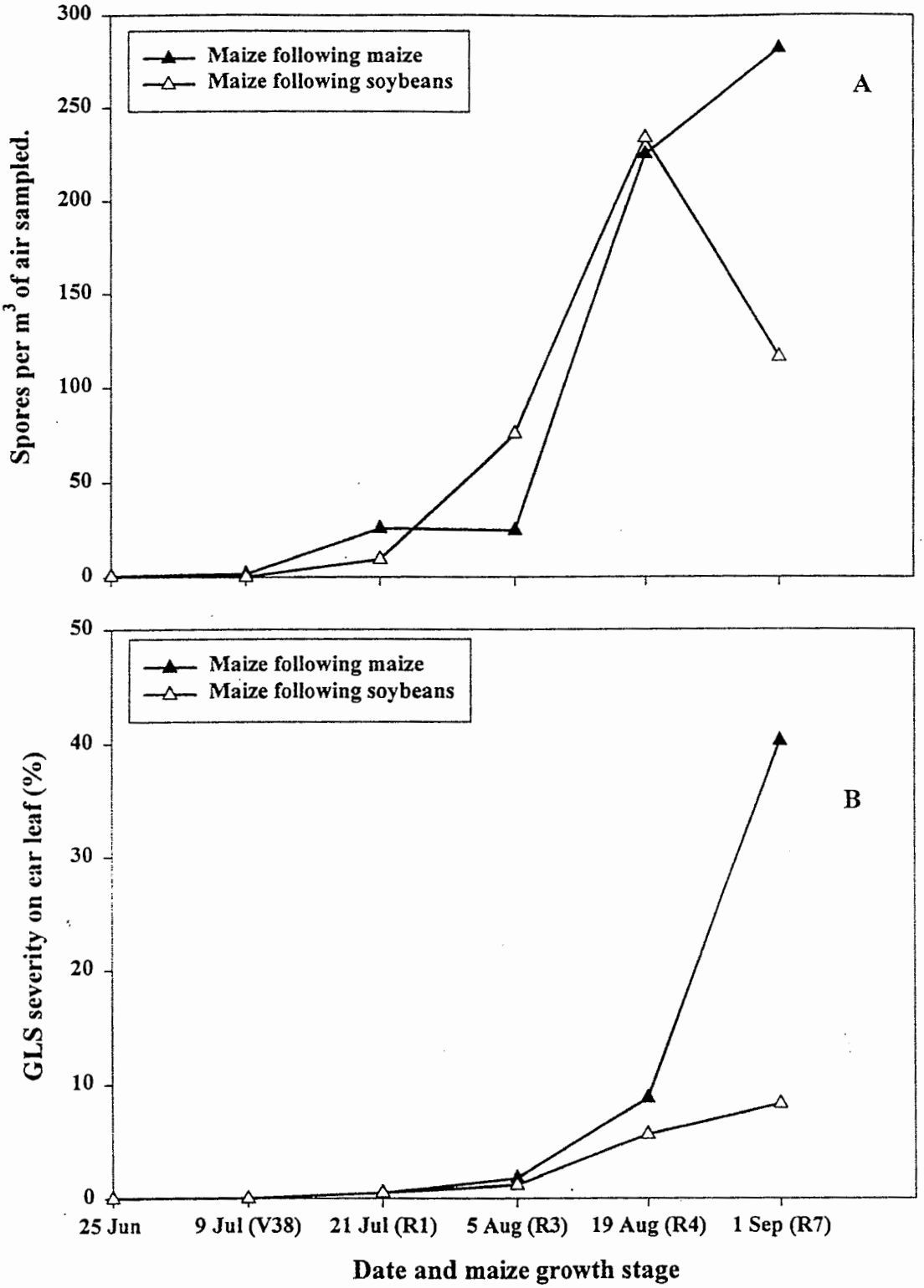


Figure 2: (A) *Cercospora zeae-maydis* spore concentrations from June to September in corn fields at the Southeast Iowa Research Farm in Iowa, in 1999. (B) GLS severity on ear leaves of maize plants at SERF in Iowa in 1999.

GENERAL CONCLUSIONS

The study described in Chapter 2 has been able to establish the quantitative relationships that exist between environmental variables , cultural factors and GLS severity. Such relationships help set the basis for future research on the development of an accurate prediction tool for forecasting GLS severity on maize. The results from this research have shown that disease can be predicted as early as two weeks before silking (R1) with a reasonable amount of accuracy. The existing relationships are based on linear regressions but it might very well be possible that a non-linear regression or neural network modeling may provide a more accurate quantification of the relationships of the several variables to GLS severity. It is also important to collect more years of data and incorporate these into the prediction model in order to be able to make predictions regarding fungicide application decisions in the field.

With regard to the study described in Chapter 3, it supports the use of crop rotation as a management technique for GLS even in areas where the disease is endemic and surface maize residue is in adjacent fields.

APPENDIX: ADDITIONAL TABLES AND FIGURES

Appendix Table 1: Deviations in response times of wetness data-loggers to (A) dew dry-off, and (B) dew onset

A

Dew dry-off

Sensor	Deviation from visual observation (mins) 6-Aug-99	15-Sep-99
A	45 min early	15 min early
C	-	15 min early
D	-	75 min early
E	75 min early	-

B

Dew onset

Sensor	Deviation from visual observation (mins) 13-Sep-99	15-Sep-99
A	10 min early	0 min
B	50 min early	15 min early
C	60 min late	15 min early
D	-	15 min early

Appendix Tables 2 and 3 : Linear stepwise regression equations from combination 1 and combination 2 respectively, showing the R^2 values and the variables that were significant at p=0.15.
Only 1998 data used.

Combination 1: MAT, RAT, RES, PLANT, DIST, RN, RH95, T, W

Period	R^2	Significant variables in regression equation
1	62%	D3 = -200.99 – 3.11 RAT + 2.01 PLANT – 0.77 DIST – 0.06 W1 + 1.39 RN1
2	61%	D3 = -179.76 – 3.02 RAT + 1.66 PLANT – 0.48 DIST – 0.11 W2 + 0.07 RH952
3	62%	D3 = -113.55 – 3.04 RAT + 1.27 PLANT – 0.43 DIST – 0.13 W3 – 2.08 RN3 + 0.07 RH953
4	60%	D3 = -212.02 – 3.39 RAT + 1.99 PLANT – 0.71 DIST – 0.10 W4 + 1.75 RN4

Combination 2: Combination 1 variables, and also D1

Period	R^2	Significant variables in regression equation
1	64%	D3 = -162.35 – 2.57 RAT + 1.75 PLANT – 0.58 DIST – 0.05 W1 + 3.59 D1
2	64%	D3 = -160.82 – 2.32 RAT + 1.52 PLANT – 0.43 DIST – 0.10 W2 + 0.06 RH952 + 3.57 D1
3	62%	D3 = - 78.34 – 2.18 RAT + 1.04 PLANT – 0.44 DIST – 0.10 W3 + 4.29 D1
4	60%	D3 = -212.02 – 3.39 RAT + 1.99 PLANT – 0.71 DIST – 0.10 W4 + 1.75 RN4

Appendix Tables 4 and 5: Linear stepwise regression equations from combination 3 and combination 4 respectively, showing the R^2 values and the variables that were significant at p=0.15.
Only 1998 data used.

Combination 3 (Table 4): TDV replaces RH95 and T, MAT, RAT, RES, PLANT, DIST, RN, W, D1

Period	R^2	Significant variables in regression equation
1	65%	D3 = -183.14 – 2.44 RAT + 1.72 PLANT – 0.66 DIST – 0.06 W1 + 0.03 TDV1 + 3.33 D1
2	62%	D3 = -153.20 – 1.99 RAT + 1.49 PLANT – 0.49 DIST – 0.10 W2 + 0.05 TDV2 + 4.92 D1
3	63%	D3 = -110.73 – 2.06 RAT + 1.23 PLANT – 0.48 DIST – 0.12 W3 + 0.04 TDV3 + 4.28 D1
4	62%	D3 = -195.31 – 3.52 RAT + 1.81 PLANT – 0.81 DIST – 0.12 W4 + 0.07 TDV4

Combination 4 (Table 5): Combination 3 variables, but no D1

Period	R^2	Significant variables in regression equation
1	64%	D3 = -221.65 – 2.91 RAT + 1.96 PLANT – 0.85 DIST – 0.07 W1 + 0.04 TDV1 + 1.35 RN1
2	59%	D3 = -203.16 – 2.91 RAT + 1.87 PLANT – 0.71 DIST – 0.11 W2 + 0.06 TDV2 + 2.31 RN2
3	63%	D3 = -104.23 – 2.89 RAT + 1.21 PLANT – 0.45 DIST – 0.13 W3 + 0.10 TDV3 + 3.83 RN3
4	62%	D3 = -195.31 – 3.52 RAT + 1.81 PLANT – 0.81 DIST – 0.12 W4 + 0.07 TDV4

Appendix Tables 6 and 7: Linear stepwise regression equations from combination 5 and combination 6 respectively, showing the R^2 values and the variables that were significant at p=0.15.

Combination 5: MAT, RAT, PLANT, RN, RH95, T, W, and RES – No DIST

Period	R^2	Significant variables in regression equation
1	49%	-121.25 – 3.26 RAT + 0.27 RES + 1.31 PLANT – 0.03 W1 – 1.52 RN1
2	58%	-125.56 – 3.22 RAT + 1.18 PLANT + 0.11 RH952 – 0.08 W2 – 5.36 RN2
3	50%	-145.74 – 2.82 RAT + 0.30 RES + 1.23 PLANT + 0.05 RH953 – 0.09 W3
4	45%	-148.89 – 3.26 RAT + 0.32 RES + 1.26 PLANT – 0.04 W4

Combination 6: MAT, RAT, PLANT, RN, RH95, T, W, and DIST – No RES

Period	R^2	Significant variables in regression equation
1	62%	-200.99 – 3.11 RAT – 0.77 DIST + 2.01 PLANT – 0.06 W1 + 1.39 RN1
2	61%	-179.76 – 3.02 RAT – 0.48 DIST + 1.66 PLANT – 0.11 W2 + 0.07 RH952
3	62%	-113.55 – 3.04 RAT – 0.43 DIST + 1.27 PLANT – 0.13 W3 + 0.07 RH953 – 2.08 RN3
4	60%	-212.02 – 3.39 RAT – 0.71 DIST + 1.99 PLANT – 0.10 W4 + 1.75 RN4

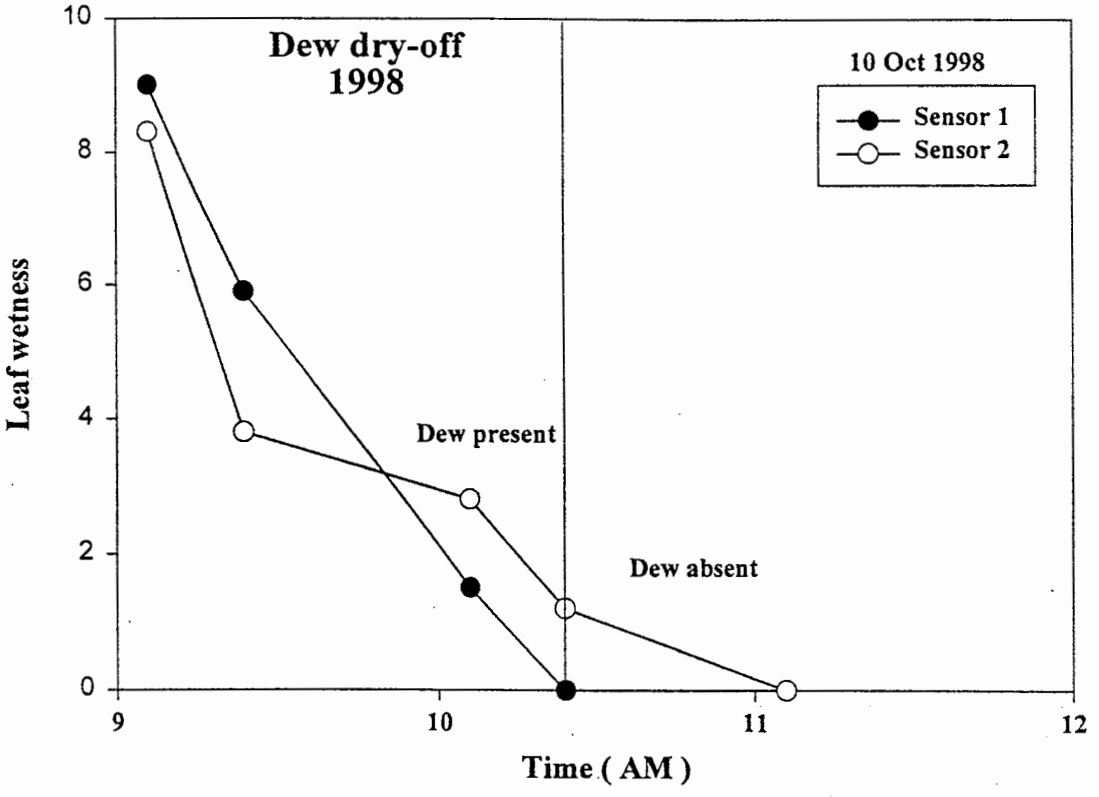
Appendix Tables 8 and 9 : Linear stepwise regression equations from combination 7 and combination 8 respectively, showing the R^2 values and the variables that were significant at p=0.15.
Both 1998 and 1999 data was used.

Combination 7: TDV replaces RH95 and T, and MAT, RAT, RES, PLANT, DIST, RN, W, and D1

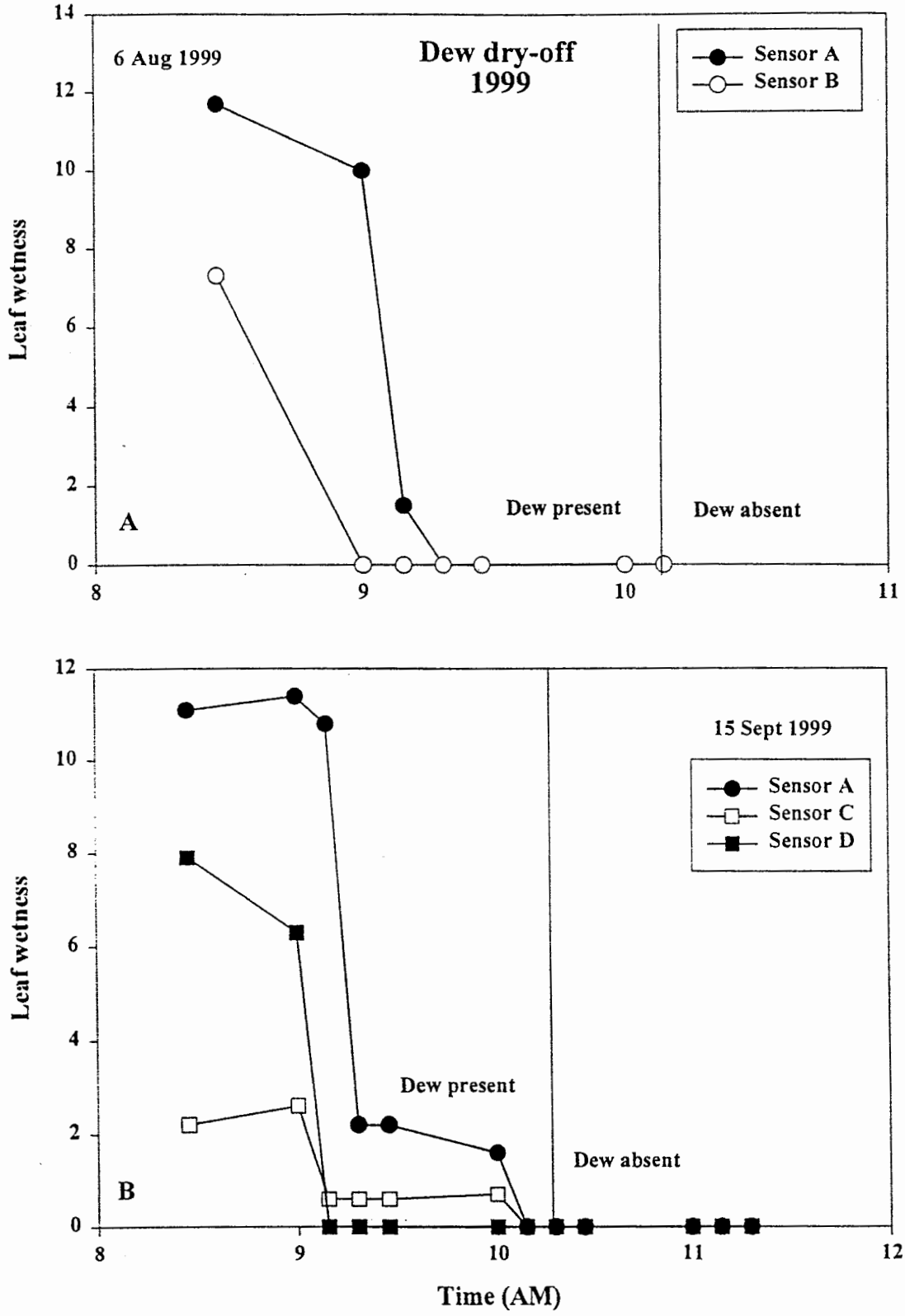
Period	R^2	Significant variables in regression equation
1	55%	D3 = -101.14 – 4.54 RAT + 0.34 PLANT + 0.44 RES + 0.04 DIST – 0.02 W1 + 0.13 TDV1 – 2.07 RN1+ 2.46 D1
2	51%	D3 = -76.13 – 4.76 RAT + 0.57 PLANT + 0.27 RES + 0.08 TDV2 – 2.80 RN2 + 2.37 D1
3	50%	D3 = -89.74 – 4.97 RAT + 0.76 PLANT + 0.35 RES – 0.04 W3 + 0.11 TDV3 – 4.22 RN3 + 1.94 D1
4	48%	D3 = -100.42 – 4.90 RAT + 0.86 PLANT + 0.37 RES – 0.03 W4 + 1.67 RN4 + 2.18 D1

Combination 8: Combination 7 variables without D1

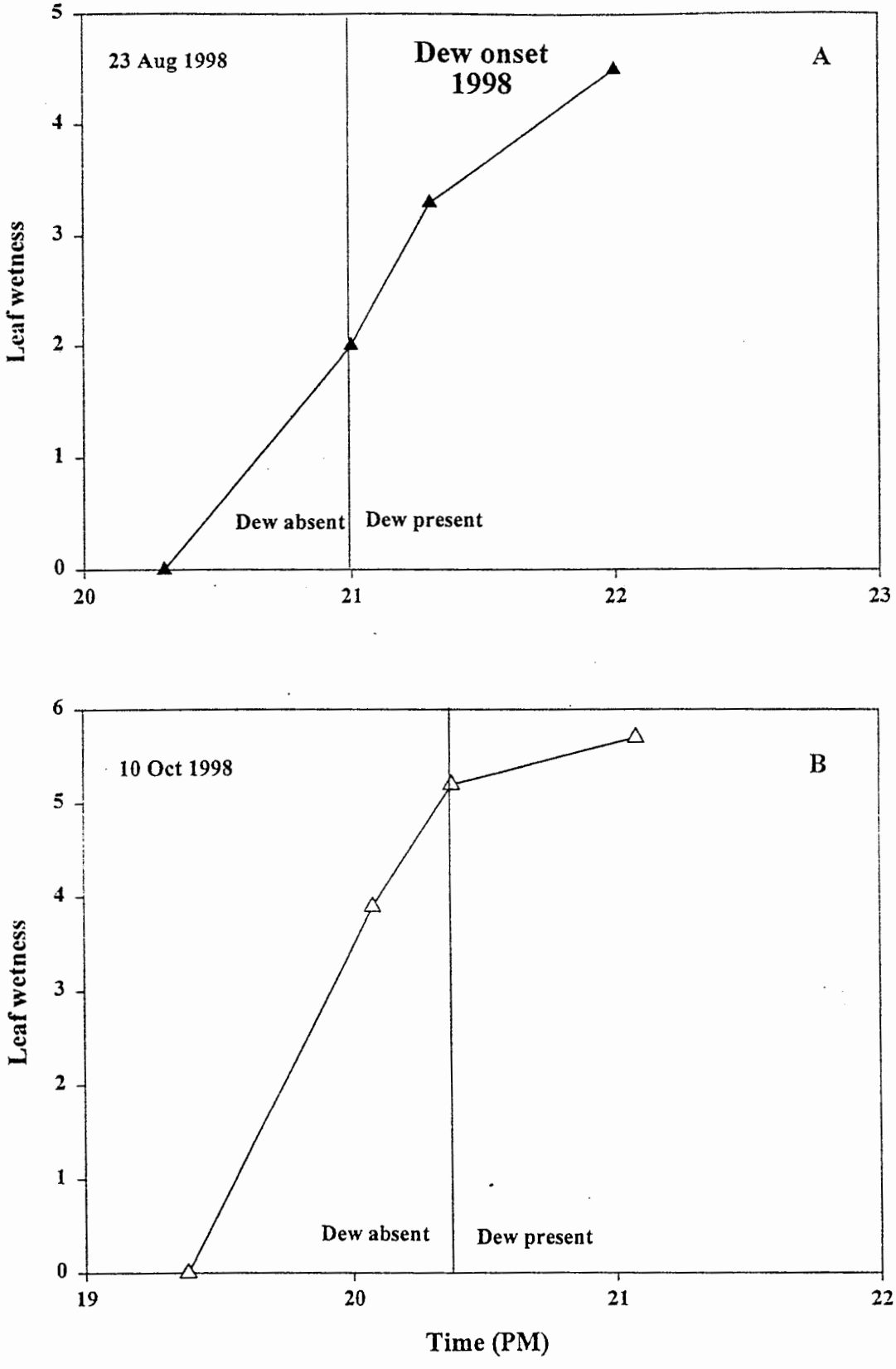
Period	R^2	Significant variables in regression equation
1	48%	D3 = -104.37 – 5.11 RAT + 0.44 PLANT + 0.53 RES + 0.04 DIST – 0.02 W1 + 0.11 TDV1– 1.96 RN1
2	45%	D3 = -97.08 – 5.31 RAT + 0.68 PLANT + 0.40 RES + 0.08 TDV2 –1.86 RN2
3	46%	D3 = -91.27 – 5.35 RAT + 0.78 PLANT + 0.42 RES – 0.04 W3 + 0.11 TDV3 – 4.63 RN3
4	43%	D3 = -109.14 – 5.36 RAT + 0.94 PLANT + 0.47 RES – 0.03 W4 + 1.43 RN4



Appendix Figure 1: Comparision of wetness readings by data-logger with visible observations of dew dry-off from maize leaves in 1998.



Appendix Figure 2 : Wetness datalogger responses to dew dry-off compared to visible dew seen on maize leaves in 1999 on 6 Aug (A) and 15 Sept (B).



Appendix Figure 3: Wetness datalogger responses to dew onset compared with visible dew onset seen on maize leaves in 1998 on (A) 23 Aug, and (B) 10 Oct.